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# DEVELOPMENT AND NUTRITION OF THE EMBRYO, SEED AND CARPEL IN THE DATE, PHOENIX DACTYLIFERA L.

BY FRANCIS E. LLOYD.

## PURPOSE AND SCOPE OF THE STUDY.

The work here reported was begun in 1907 upon my appointment as Cytologist to the Agricultural Experiment Station, Tucson, Ariz., in connection with the special investigations on the date which have there been carried forward during recent years, more especially by Dr. A. E. Vinson. The material was collected, through the co-operation of Dr. Vinson, at the Station Date Orchard, at Tempe, Ariz. At the inception of the work it was my purpose to study exhaustively the whole period of embryogeny with reference to the rôle of the various foods and other materials in the seed and carpel, and for this purpose, it would have been necessary to make use of both preserved and fresh material. This object was defeated by my removal to Mexico, and I was therefore compelled to make use exclusively of preserved material, with the exception of some of the earlier stages which I studied before leaving Tucson. In consequence, the sugars have, I regret, been left out of account. This is of less consequence as regards the carpel, as they have been studied, by the methods of the chemical laboratory however, by Dr. Vinson, who has embodied his results in various papers to be later referred to. The present paper records my studies therefore of the anatomy and histology of both seed and carpel from the developmental point of view, and of the rôles of tannin, starch, oil and reserve cellulose. Material of two well marked races, Rhars and Deglet Noor, invert and cane sugar types,[1] respectively, has been examined, but it has developed that such differences as exist are, from the present point of view, negligible. When desirable, I have noted such differences.

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[1] Vinson, 1906.

The date seed has long furnished material for studies begun with Malpighi, later continued by Sachs, whose work is fundamental, and followed by several others whose work will be mentioned beyond. This has been due in part to the ease of obtaining material and the conspicuousness of the phenomena during germination. In the development of our knowledge of enzymes it has played an important part, not the least important contributions in this field having been made by Americans. The positive evidence thus gained is of value not only in interpreting what goes forward during germination, but also during embryogeny. Working backward from the resting period, at which point the studies available up to the present time have begun, we are able to understand many details for which otherwise only speculative explanation could be advanced.

## Methods.

Material was gathered on alternate weeks, from the time of pollination till maturity, from one of the two races and preserved in three series, one in a watery solution of copper acetate, as recommended by Strasburger, one in chrom-acetic fluid, followed by alcohol, the third in alcohol-acetic, (2:1). For examination, free-hand sections were used, except in earlier stages, in which it was necessary to use microtome methods for the determination of minutiae of structure. Free-hand sections were treated with iron salts, (ferric acetate or chlorid).

After long standing in copper acetate, the material contained, in many cases, a considerable amount of metallic copper, either within the tissues, on its surface or in the fluid. This was evidently due to the reduction of the copper salt, chiefly, it seems probable, by the invert sugars present. The possible source of error, due to the presence of tannin, was excluded by the use of ethyl nitrite, on a small but satisfactory series of material prepared for me during the spring of 1910 by Dr. Vinson at my request. This check material showed that my observations on the copper acetate preparations were correct. This is of considerable importance during the development of the endosperm, where the fixation seemed to lead to misinterpretation.

Similarly the long exposure to copper acetate of material containing oil results in the formation of a white, apparently amorphous deposit in certain situations. In a few preparations, under conditions which I am unable to determine exactly, beautiful dendritic masses of minute crystals have appeared. In others, large numbers of pale green sphaerocrystals have been found on cutting a section, which had the appearance of being an oil-copper compound. Their insolubility in suitable solvents seems to preclude this interpretation. Nevertheless, these should be understood in order to exclude completely sources of error, as it is not at all unlikely that where oil is exposed to copper salts, the oil would be reduced in amount. In the present case, the alcoholic material was used as control.

I am obliged to Professor R. H. Forbes and Dr. A. E. Vinson for much assistance in the obtaining and preservation of material, and to my colleagues, Professor B. B. Ross and Professor C. L. Hare, for criticisms from the chemical point of view of certain interpretations. This study, begun at the Arizona Agricultural Experiment Station, has been largely prosecuted at the Alabama Agricultural Experiment Station.

## STRUCTURE AND DEVELOPMENT.

Organogeny of the fruit. Some account of the development of the parts of the fruit will be necessary in order to make evident the anatomical and histological changes which take place during the time between pollination and the final maturation of the fruit. The materials have been examined, not with the object of studying the cell-to-cell minutiae of the embryology, but rather to follow the main outlines of development of the embryo, seed and fruit, together with their nutritive inter-relations discoverable with the methods at hand.

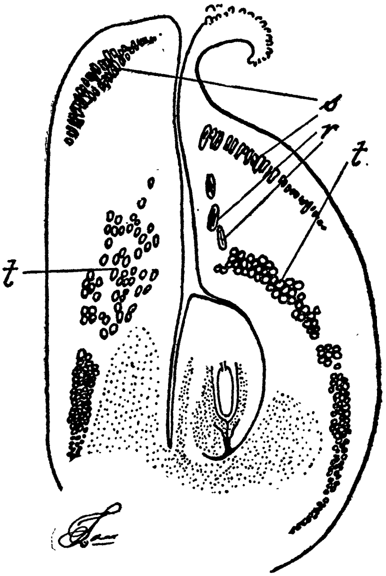
The whole extent of the development of the date fruit falls rather naturally into three periods: a, that extending from the time of pollination (Stage I, f. 2) until the endosperm ends its pavement phase of development (Stage II, f. 7); b, that between this time, and the final closure of the endosperm cavity (Stage III, f. 14); and c, the period following until maturation (Stage IV). It will be convenient for cross reference to speak of these stages and periods. Period I occupies about eight weeks. The young seed is then 4 mm. long by 2 mm. in diameter. The whole fruit measures 7.5 by 7 mm. (broad). Period II occupies about three to four weeks. The fruit is 9.5 mm. broad by 10 mm. long; the seed 7 mm. long by 2.5-3 mm. broad. It is relatively a very short period and one of rapid change, characterized by the development of the endosperm, and marked topographic changes in the ovule in general. The third period occupies a period, following the closure of the endosperm cavity, covering about 15 weeks, and is characterized by the development of the embryo, which, until the end of period II, remains very small.

### Stage I. Beginning of the First Period.

The pistil (f. A), with the exception of the stigma, is enclosed in the involucre. Three pistils are present, only one of which persists normally.

*Carpel.* The whole pistil at the time of pollination measures about 3 mm. in length by 1.8 mm. broad. The dorsiventral diameter (1.2-1.4 mm.) is somewhat less. The locule is small, and completely filled by the anatropous, central basal ovule. The short style is traversed by a canal lined by secretory cells—pollen tube guiding tissue. The canal is continuous with a groove, which at the upper part of the ovule is single, but bifurcates as it passes downward, one groove passing on each side of the funicle (f. 2-2e). In front of the micropyle the glandular tissue spreads out to form a cap over the exostome (f. 2). These grooves persist and enlarge, and may be traced (f. 11) throughout the whole development of the fruit.

At this time, with the exception of the epidermis of columnar cells, external and internal (endocarp), there is to be recognized only the tanniferous layer of idioplasts. Within a week after pollination, active differentiation of tissues has commenced at the apex of the carpel, which begins to project beyond the bracts. The epidermis is thus more or less exposed and is becoming strongly cuticularized. Hypodermal tannin cells are evident here and there, and the layer of stone cells is rapidly forming. Within the mesocarp, numerous raphide bearing cells have been formed, and the tannin-idioplasts are more strongly developed.



A. PHOENIX DACTYLIFERA.

Longitudinal section through pistil at approximately the time of pollination. The distribution of starch is shown by stippling.—*t*, Tannin idioplasts. *s*, Stone cells. *r*, Rhaphide idioplasts. The stylar canal is seen.

*Ovule.* The ovule at the time of pollination presents several matters of interest from the present point of view. Of the nucellus nothing remains but a cap of a single layer of cells, in a state of rapid disintegration (f. 1). The bulk of the ovule is of nucellar origin (f. 2, 5), and, after the disappearance of the nucellus, may be regarded as a chalazal tissue. It thus comes about that only the upper end of the embryo-sac is surrounded by the integuments, which form a crown resting on the top of it (f. 3). The micropyle is evident, and is lined by actively glandular cells, the inner superficial cells of the inner integument. As seen by the figure, the endostome juts forward into the exostome, which is open. The anatomy of this region suggests the explanation that the glandular tissue of the carpel facing the exostome is a center of attraction for the pollen tube till it reaches this point. The secretion, which is doubtless thrown out by the glandular cells of the endostome, then exerts a superior attraction perhaps quantitatively only, or, it may be, qualitatively, and this leads the pollen tube to enter the exostome, which is its normal course. I have elsewhere dealt with the problem of the direction of the pollen tube (Lloyd, 1902) suggesting that the pollen tube is guided in its course by the differential distribution of a stimulant arising from the egg-apparatus, chiefly the synergidae, and that the whole phenomenon is chiefly a chemical one. The evidence here before us prompts a modification of the above suggestion, which however does no violence to its fundamental feature, to the effect that the stimulant may be handled in a system of relays, the pollen tubes being guided from one relay to the other. In the date the relay stations, so to speak, are in the carpellary guiding tissue, the glandular endostome tissue and the egg-apparatus and egg cell itself. There is no objection to the assumption that these offer either a renewed stimulus of the same kind, or even a different kind of stimulus each time, in view of the work of Lidforss, who showed that positive curvatures are shown by pollen tubes toward nineteen proteins of various groups.[2] It appears not improbable that refined methods may discover that where different guiding tissues occur, each one involved produces its specific secretion which restimulates the pollen tube from time to time on its course.

The innermost layer of cells of the inner integument, already mentioned, also presents a special degree of activity. The cells become deeply columnar and distended at their free extremities where they touch the embryo-sac. This layer of cells is clearly a tapetum, and is analogous to that described for the Compositae[3] and for a number of other plants by various later authors.

This tapetum is contributed to, to some extent, by the adjacent chalazal cells, so that it extends some distance down the embryo-sac, and further down on the funicular aspect, where it reaches as far as the antipodal region. Here it has a distinctly pronounced development.

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[2] Lidforss, 1909.

[3] Goldfluss, 1898–9.

At this moment, namely at pollination, or very soon after, the antipodal end of the embryo-sac shows a remarkable amount of activity. This is seen especially in the very irregular and rapid backward extension of the endosperm, previous to the division of the endosperm nucleus, leaving the antipodal portion of the embryo-sac in its original position (f. 3, 5). There is formed in this way a curious several-armed chalazal extension which, in view of the digestion of the tissues in its path[4] must be regarded as a haustorium. Its function is the same, I believe, as that of analogous haustorial structures formed by the endosperm in *Plantago*,[5] in which, however, they are more highly specialized in form. Its total activity, when followed through the whole course of events, is relatively very great, as will appear from its position and volume in the ripened seed.[6] The observed phenomena relating to this activity are recorded beyond. At this point it is sufficient to say that, in a week after pollination, the amount of development is quite marked, there being several cul-de-sacs penetrating deeply into a tissue heavily loaded with tannin. At the same time there is evident the beginning of that torsion which ends finally in the complete displacement of the embryo (f. 5).

### Stage II. Close of the First Period.

*Carpel.* The epidermis is strongly cutinized. The hypodermal parenchyma cells are still cubical or rounded, but show the accumulation of tannin. The layer of stone-cells is completely developed, as also the tannin-idioplast layer. Tannin-idioplasts occur also throughout the mesocarp in the sutural sector (f. A, 7, 11), and in all parts of the basal region. They are especially numerous near the funicle. Morphologically, this is placental tissue, and is constantly characterized by scattered tannin cells. Below the insertion of the funicle, there are two longitudinal grooves, one on each side of the ovule, which may be called sutural sulci. These arise as the continuations of the stylar canal, and are earlier functional as pollen-tube guiding grooves already described. Above the funicle the sulci unite and have a common meatus, finally becoming single. The epidermal (endocarp) lining of the sulcus has the appearance of glandular tissue, and simulates the nectar grooves found in certain plants (e. g., Liliaceae). There is a greater amount of tannin in them, in common with the tissues of the placental region than elsewhere in the endocarp. There is, however, no indication of glandular activity. The sulci, as a result of growth pressures, are secondarily more or less irregular in their disposition, as is seen from the examination of a series of sections.

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[4] At first similar in appearance to the irregular cavity formed in the pine nucellus by the growing pollen tubes.

[5] Balicka-Ivanovska, 1899.

[6] It is evident that the chalaza in the mature date seed is not primary. Its developmental continuity, however, is clear and so we may properly call it the chalaza.

Raphide cells are now relatively much less numerous, though they occur in scattered positions throughout the mesocarp.

The endocarpal epidermis is so far differentiated that the cells are considerably elongated and irregular in tangential contour.

*Seed* (f. 7). The torsion begun during the first week (f. 5) has progressed so far that the embryo is now one-third of the entire length of the ovule distant from the original position. A corresponding amount of torsion has been experienced by the upper end of the ovule, so that the backward extension of the endosperm, beyond the chalaza, is well started. The endosperm is parietal, and of a single layer of cells. The tanniferous tissue about the chalaza is very pronounced but is not readily distinguishable on account of the general tannin reaction. The chalazal cul-de-sac has enlarged, and continues to do so beyond this period, so that in the definitive seed its size is marked.

The torsion which the integuments undergo is a differential one. The epidermal cells move relatively less, while the greatest movement is found in the innermost layer of cells. Thus it happens that the exostome of the micropyle does not change position as much as the endostome, which retains its topographic relation to the embryo. As a condition of further growth, there is yet but little differentiation in the integuments. There is growth in thickness and extent, but the cells retain their undifferentiated character.

### Stage III. Close of the Second Period.

*Carpel.* Aside from the extension of the tissues by growth, there is little to record, except only that, as a result of the mutual pressure of tissues, the inner zone of the carpel becomes slightly compressed. A small amount of disorganization is apparent here and there, foreshadowing the shining fibrous threads regarded as a part of the endocarp. The crushing is more apparent at the placental region than elsewhere, and it is especially noticeable in its effects upon the placental sulci, which become distorted. The contingent cells within the sulcus become denser in character and there appears as it were a mucilaginous thickening of some of the cell walls, especially near the meatus. There are numerous tannin cells of idioplastic nature, though the tannin is not wholly confined to these.

*Seed.* In the seed the greatest change is in the inward growth of the endosperm, and its extension backward beyond the secondary chalaza. The centripetal growth proceeds at first at the chalaza (f. 10) filling the cul-de-sac; the inwardly moving walls then meet at the upper end, the fusion progressing toward the opposite (micropylar) pole of the embryo sac. All nutritive changes in the endosperm progress, similarly, from the chalaza toward the micropylar pole. When the space is entirely filled, and before secondary changes set in, the endosperm cells are very thin walled, with the nucleus suspended in the middle of the cell by numerous radiating protoplasmic threads. They are isodiametric, but their radial measurements begin to increase rapidly, concurrently with the later growth of the seed. The growth of the integuments is very rapid, in view of the rapid increase in volume of the seed, and their definitive characters are still absent.

The embryo at this time is spherical in form, 60-75 microns in diameter; at the time when the cavity is just obliterated, the seed measures 2.5 mm. in diameter in the transverse plane in which the embryo lies. The increase from this time, to a transverse diameter of 8.5 mm., in the ripe seed; and from a length of 8-9 mm. to 28-30 mm., is the period of maturation, anatomically speaking, during which the greatest changes are to be found in the endosperm and embryo. There is usually still more torsion, which brings the embryo into the ultimate position, about midway the seed. The position is, however, variable and may occasionally be quite abnormal. From the circular embryonic area, visible on the outside of the seed, a slender line may be traced downward. This is the evidence of the torsion, and is the anatomical indication of the micropyle.

### Stage IV. Close of the Third Period.

*Carpel* (f. 32, 33). The definitive epidermis, 15 microns deep, is heavily cutinized, the cuticle 4 microns thick. The cells are nearly isodiametric and straight walled. There are occasional stomata, each supported by four or five accessory cells. Beneath the epidermis is a thin layer of parenchyma 45 microns thick, of four to five layers of tannin cells, compressed radially. Within this layer again is the zone of stone cells, occupying a very irregular thickness (60-120 microns) according to the position of the individual cells. The longer ones are placed radially. It will be convenient to regard these tissues as constituting the exocarp. The mesocarp is composed of very thin-walled parenchyma, with here and there a raphide, or tannin idioplast, and penetrated longitudinally by vascular strands. The midrib of the carpel is marked by a median vascular strand, and the suture by a dipping in of the layer of stone cells (f. 11). The inner zone of the mesocarp is composed of more or less crushed and disorganized parenchyma, which, in the ripe fruit is conspicuous as loose, shining, fibrous masses (the "rag"). These are only loosely attached to the endocarp, and constitute an irregular, poorly delimited zone which must properly be regarded as part of the mesocarp. The endocarp is a definite, rather tough membrane (f. 34), composed of the inner epidermis and more or less underlying tissue of compact cells. This membrane adheres somewhat firmly to the seed but splits readily longitudinally on removal.

Along the side of the placental ridge there are two parallel strands of endocarp epidermis which are especially obvious in chromic acid and ethyl nitrite material. They appear to be differentiated on account of their anatomical relation to the underlying placental tissue which is rich in tannin. It is possible, therefore, that they represent transfusion areas, where the tannin passes from the carpel into the ovule.

*Seed.* The outer integument (f. 31). The epidermis is completely sclerosed, having pitted walls. The cells are, on the whole, elongated parallel to the axis of the seed, but at the same time they show *en face,* a very great deal of torsion and irregularity. There is an irregular hypoderm of more or less similarly sclerosed cells, beneath which are thin-walled tannin cells. It may be an expression of physiological correlation that tannin is not to be found in the sclerosed cells. The inner integument is somewhat difficult to delimit sharply; it consists of at least two layers of cells, the outer and inner epidermis, with usually one or two additional layers of compressed cells between. The entire integumental covering measures about 30 microns in thickness, but thickens toward the raphe and above the embryo.

Directly impinging upon this, and within it, is the thick-walled endosperm. The outermost cells are very frequently isodiametric, are thinner walled than those within, and have few pores or none. The longer ones have pores at their inner ends, or on the sides when unusually long. All the cells radiate toward the morphological middle of the endosperm, which is marked by a mass of isodiametric, roughly spherical cells.

The embryo is short and cylindrical in form, but is frequently distorted longitudinally. It is placed at right angles to its original position. The end toward the axis of the seed is the haustorial cotyledon, with which numerous students, beginning with Malpighi and Sachs, have familiarized us, and may be somewhat enlarged, when seen in a longitudinal section of the seed. In transverse section, this end of the embryo is usually smaller than the radicular end. The whole embryo measures about 2 mm. in length, of which one-fifth is the hypocotyl. The radicle proper is scarcely .3 mm. long.

The form of the seed, as seen in transverse section, varies with the race. In Deglet Noor, the outline is nearly circular, but may be disturbed by a low, lateral ridge. This is very marked in Rhars, so that the outline is lobular.

## OCCURRENCE AND DISTRIBUTION OF STARCH, TANNIN AND OIL.

### STARCH.

Starch is to be observed, in the form of transitory grains, only during a relatively brief period of the earlier developmental stages. Its appearance at all seems to be connected with the slower rate of growth prior and immediately succeeding pollination. During the later period of rapid development of the fruit, none appears at any time.

### Observations.

*Nondescript.* May 16, unpollinated. Starch in the upper zone of pedicel, just beneath the receptacle, in large grains. Also in the inner (ventral) part of the parenchyma of the outer bracts, and in all the parenchyma of the inner bracts toward their bases, and in the ventral moiety in the basal half. In the receptacle below the insertion of the pistils, but much less in a zone above the pedicel. Pistil: Rather abundant in the basal part, becoming reduced in quantity in the upper half, where it is of very minute grains. Similarly minute grains in the ovule, in the funicle, outer and especially in the inner integument. The amount of starch is greater near the nucellus, becoming reduced to none toward the periphery. There is no starch in the tannin idioplasts in the carpel or elsewhere.

The same material one day after pollination showed the same distribution of starch.

*Tronja.* May 16. One day after pollination gave similar results qualitatively but much less marked.

*Deglet Noor.* Pollinated, April 6; fixed, May 16. Starch in a few scattered grains in the pedicel in the parenchyma adjacent to the bundles, in the parenchyma at the base of the carpel, and in the inner integument between the funicle and micropyle.

*Nondescript.* Pollinated, Apr. 11; fixed, May 28. Starch in the ovule restricted to the basal part of the funicle and to inner zone of outer integument.

*Deglet Noor.* Pollinated, April 6; fixed, May 16. Starch restricted to the mass of tissue between the funicle and micropyle and to a small volume of tissue along the funicle nearby. More in the base of the carpel itself.

Up to this time the growth of the ovule has involved chiefly the chalazal half, from which concurrently the starch has disappeared, and in which it never reappears.

It is thus seen that this form of carbohydrates plays only a brief rôle during the embryological period. The deposition of starch appears to be inconsistent with a very rapid development such as characterizes the date fruits after the seventh week following pollination.

The course of the disappearance of starch seems to be connected, in part, with the general growth of the ovule, as well as with its removal into the embryo-sac.[7] The latter undoubtedly accounts for a part of it, but that which remains in the outer integument is probably consumed during the earlier phases of growth in this structure.

*Embryo.* Starch appears in the embryo at first at a comparatively last stage of development. At 17 weeks (f. 17) a very small amount is seen in the root cap and in the apex of the cotyledon. During the embryological history starch is unimportant quantitatively; during germination as described by Sachs (1862) its importance increases.

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[7] Ikeda, 1902.

## TANNIN.

One who now-a-days makes use of the blanket term tannin throws himself open to criticism. Nevertheless, it is not always possible to avoid it, especially when blazing the way into material necessitating the use of histological methods. A distinction has been drawn between "plastic" and "aplastic" tannins, and objection has been raised[8] to the use of these terms. They explain themselves, but it is obvious that we can use them only to describe tannins in a particular structure when they have been shown, in the end, to remain definitively, or to disappear during metabolism. "Aplastic" may, despite that objection, be used merely as a matter of temporary convenience to designate that tannin which appears in a particular situation to remain permanently thereafter. Conversely, plastic tannin is that which appears only to disappear. Such tannin is analogous, in appearance at least, to transitory starch, and the evidence to be offered indicates that the comparison is justified by more than appearance alone. If the attempt is not made in what follows, to distinguish chemically the tannins with which I have concerned myself, this is due to the conditions and, not less, to the point of view.

The history of the study of tannins is extensive, and the materials which have been studied profuse. Happily, Dekker (1906), in a most painstaking way, has brought the available data into the compass of a small but rich work, and for this the thanks of the botanical fraternity are due him. A perusal of the historical survey in Dekker's paper shows that there are two general views held as to the physiological rôle of tannin. These are, obviously, that tannin is a waste product, and, opposed to this, that it is related to glucose and is of use in forming more complex carbohydrates than itself. Doubtless both are true, but, doubtless also, the same substances are not meant in all cases. Thus, Sachs (1862) regarded the tannin in the germinating date seedling as an excrete, and the evidence supports his view. Contrariwise, Servettaz (1909) has observed that tannin occurs in certain situations in the young, rapidly developing ovule (in the Eleagnaceae) and argues, with equal right, that here this substance is concerned with nutrition. In view of these observations, it would seem the rational procedure to regard the tannin which accumulates, and remains permanently, in certain cells of the germinating date as different chemically from that in the young ovule of the Eleagnaceae. The chemist may tell us later that, indeed, the former is tannin, and the latter not. These two instances only are cited, because as we shall presently see, they are pertinent to the matter in hand. For the present purpose, we must be content to call those substances tannins which afford us the visible reactions which are usually resorted to, and rely upon observation to tell us whether a particular tannin is an excrete or a nutrient.

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[8] Pfeffer, Physiology of Plants. p. 493.

Aplastic tannin is known to occur in the date. Thornber[9] studied a series of date fruits from the "size of a pea until full ripeness" and came to the conclusion that there is "no general distribution of tannin" but that it is "strongly segregated in a layer of very large cells near the surface of the fruit and, especially in the younger stages, near the seed." Avoiding hypercriticism as to the meaning of "near the seed" these facts have been further mentioned and illustrated by Vinson (1910) for the purpose of demonstrating the value of ethyl nitrite vapor in the study of tannin in plant tissues. Vinson believes that "a green date may also be easily divided into astringent and non-astringent portions with a pocket knife" and has said to me personally that, before ripening, the portion (mesocarp) lying beneath the layer of idioplasts (f. 32) is not astringent, but that the contents of the idioplasts may be expelled by gentle crushing and appear mucilaginous. The inference is at hand that the tannin is confined wholly to the idioplasts, but, for reasons which will be given beyond, I think this is not quite true. Howard (1906) on the other hand concluded from his study of the ripening persimmon that before ripening the tannin is distributed in the cells of the "loose parenchyma where the tannin cells are located" as well as in the idioplasts. During ripening the tannin, according to Howard, "becomes condensed in certain specialized cells" (Bigelow, Gore and Howard, 1906, p. 702), and there becomes insoluble. The observed facts in the date (Lloyd, 1907),[10] as we shall see, do not accord with this view, though in the date as in the persimmon, either during natural or artificial (Vinson, 1909) ripening, the tannin becomes insoluble,[11] and hence the lack of astringency in the fully ripe fruit, in these as in other cases, *e. g.*, the sapodilla, according to Geerlig (1909). What is true of the specialized tannin cells, in this regard, is true also of the remaining tannin cells, those namely in the inner mesocarp and endocarp, and, also, in the integuments, since they are alike tasteless in the ripe fruit.

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[9] 1906. Not over his personal signature.

The only remaining observations on the date which touch on the following account are the following: Pond (1907) reported finding tannin in the membranes (endocarp) about the seed, and cautioned students of digestion in the germinating date against the introduction of tannin from the integuments into the fluids to be tested for reducing sugars. Sachs (1862) discovered tannin diffused through the tissues of the resting embryo, and described the final accumulation of such material in certain parenchyma cells of the seedling.

Reference has been made to Servattaz' observations on the Eleagnaceae. The tannin, *"substances tannoides,"* he says, occurs in the ovule, in the elongated cells of the nucellus at the base of the embryo-sac and connecting this with the vascular tissue of the raphe. It occurs also in the midlayer of cells in the external integument. Continuing, he says, "Dans l'ovule, ces substances ne prennant pas naissance à la suite de la destruction de l'amidon, car il ne s'y forme jamais de réserves amylacées. Le glucose abonde dan toutes les parties de l'ovule . . ." (p. 353). Servettaz, further, inclines to the belief that the tannin may be employed in the nutrition of the embryo-sac, and advances the interesting view that antipodal cells play an active rôle in the process: ". . . les tannins amenés à la base du nucelle seraient transformés en glucose par la 'cellule antipode' qui occupe l'extrémité du sac embryonnaire, car cet organe ne renferme pas de tannins . . ." (p. 411). He concludes that, though tannins occur in such situations that they must be considered a waste, he also takes the position that they are in other situations useful, and cites the finding of Gerber (1897), who has shown that tannins give rise to glucose during the maturation of some fruits.

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[10] Not over his signature.

[11] Mylius (see Dekker, 1906), appears to have made observations to the same effect, but I have not seen his paper.

In what follows it is hoped to show that (1) in the date tannin occurs in special cells in such a state that we are precluded from supposing that it serves any but secondary functions, such as protection, as shown by Vinson (1909). This tannin is aplastic. (2) That there is no condensation of tannin just prior to ripening, meaning by this a movement of tannin from the surrounding parenchyma to the special cells or idioplasts. (3) that tannin (plastic) occurs and disappears at such times and in such situations as to warrant the conclusion that it is a nutrient, and in this is analogous to starch, reserve cellulose, oil, etc.

*Carpel.* Hypodermal tanniferous zone (f. 33). The parenchyma cells lying between the epidermis and the zone of stone cells are more or less flattened radially, and take up, with the epidermis, a thickness of about 60 microns, of which the epidermis accounts for 15. In the *Rhars,* the majority of these cells, usually excluding the epidermis, contain tannin, and they constitute a zone one to three cells in thickness, immediately beneath the epidermis. Occasional cells among them are sclerosed and may also contain tannin. In *Deglet Noor* there is a similar zone, but usually of only one cell in thickness. The hypodermis of commercial dates displays the same tanniferous layer, the cells containing tannin "vacuoles," or masses lying in the vacuoles. In material fixed with a chromic acid mixture, the tannin does not appear in the vacuole in all cases, but in or against the protoplasmic lining.

In addition to the tannin in the more conspicuous tanniferous cells lying immediately beneath the epidermis, a small amount of tannin occurs in all the parenchyma to a depth of 180 to 200 microns inside the zone of stereids (f. 33). This tannin is always in the form of minute droplets scarcely ever more than 6 microns in diameters and for the most part smaller than this. The cells themselves show no peculiarities, and in no way are differentiated from the remaining parenchyma within, except in containing these droplets of tannin. Thinking, upon discovery, that they might be oil, I tested them with alkanet, with negative results. Upon allowing the section to lie over night in very weak methyl blue, they became very deeply stained. Their color reactions are identical with the more abundant tannin of the hypodermal or idioplast layers, but they are not conspicuous on account of their small size. Their occurrence does not extend as far as the idioplast layer, nor do any of the parenchyma cells near to this layer show the presence of tannin droplets.

Origin—Indications of this tannin zone are to be seen after pollination. One week thereafter, distinctly differentiated hypodermal columnar or cubical tanniferous cells may be seen, though as yet the zone is not continuous. At the time of pollination, these cells are not yet differentiated, but the epidermis shows a diffused tannin reaction. With the dying back of the stigma and very short style, the tannin cells appear rapidly. These cells, constituting the hypodermal tannin zone, are sharply differentiated once for all, and differ from the idioplasts only in their lack of special structural character. They are distinguishable only by their contents.

Sub-hypodermal tanniferous zone (f. 32). This zone is composed of large idioplasts (giant cells[12]) and is of varying thickness (1 mm. more or less), lying about 1 mm. below the surface of the carpel.[13] The layer is four to six cells deep, roughly speaking, in Rhars and Deglet Noor, the number of the cells depending on the size of the elements as well as upon the number which may be counted in radial direction. Tannin similar to that in these cells occurs also in the integuments of the seed, and in the mass of tissue, partly of integumental, of chalazal and of funicular origin, which fills the deep prominent groove characteristic of the date seed.

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[12] So called by Swingle, according to Howard *l. c.*

[13] The character of the zone varies considerably with the race. A few of the difference are illustrated by Vinson, 1910.

Origin of the layer of tannin idioplasts. This layer makes its appearance at a very early stage of development, some time before pollination. For the present purpose it is sufficient to sustain my contention to show its condition prior to pollination and its development thereafter. Both fresh and preserved materials including nitrous ether preparations, were studied.

At this time the layer in question is continuous from the base of the carpel, upwards, overarching the ovule. At the base the component elements are nearly or quite spherical, and about 15 microns in diameter. Toward the base of the style, where the elements are the largest, they reach a size of 45-60 microns. The arch is interrupted at the apex by the stylar canal and its immediately surrounding tissues, and here also the thickness of the layer of four to six cells is the greatest. Toward the base of the carpel, it dwindles to the width of a single cell. The layer is homologous with the similar tissues in the bracts surrounding the three pistils, and appears the same physiologically. Precisely the same condition is found in *Chamaerops humilis*. Tracing them through the succeeding stages of development, there is no change in the constituent elements, except an increase in the amount of their tannin content. Other cells are added to the tissue, being intercalated between those already present, or in a radial direction.

The contents of the cells either lie in a layer about the wall, or partially fill the lumen, and are highly vacuolated,[14] this, as we shall presently see, in contrast to the same cells later on. In material which has not been prepared with a view to the detection of tannin, they offer no points of striking contrast so that they may be distinguished from their neighboring parenchyma only by their size, and in this respect resemble the primordia of the stereids, which lie, also in a layer, between the tannin cells and the epidermis. Both the former and the stereid primordia are parenchyma cells which become secondarily specialized, and both have this in common also, that once their peculiar content is laid down, it remains unchanged: namely, in the tannin cells, tannin of the fixed variety, and in the stereids, cellulose and lignin.

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[14] Due perhaps to the liberation of gas during the reaction.

What appears however in this condition as a simple peripheral layer of protoplasm, must be in a living condition, as shown by the proper reagents, protoplasm imprisoning a solution of tannin. Alcohol acetic material, even after prolonged treatment, does not seem to be depleted of the tannin, for upon the addition of methyl blue, a fine, flocculent precipitate is discoverable, occupying the whole of the interior vacuole. Material treated with copper acetate displayed a variety of curious forms of solid tannin-copper compound, and these are, I believe, to be regarded as artefact. Thus, sometimes a coarser or finer network of tannin forms a sponge-like mass, filling the interior; or into this may extend, from a solid, continuous lining, arms of the same; or the whole interior may be filled with a vacuolated mass; or finally it may appear as a complete, homogeneous lump filling the whole of the cell as seen in ethyl nitrite material, as well as in that prepared by other reagents. In these conditions, one may hardly discover the protoplasm which is responsible for the secretion from this, and for this purpose copper acetate, with subsequent treatment with other metal salts for color effect, is really inadequate. Much better is methyl blue in this regard.

Not however to do the copper acetate method too great injustice, I may add that sometimes a quite fine precipitate is to be seen, though the protoplasm is still not to be successfully differentiated to the eye.

To return to the anatomy of the tannin idioplast layer.

While generally continuous, and forming a zone of fairly uniform thickness there is, along the line of carpellary fusion, a scattering of the cells. Single cells, or groups of these occur in all the tissue between the inner limit of the carpel, and the outer limit of the zone in question (f. 11). In the condition before pollination, this dipping inward of the tannin tissue is not obvious, for the reason that there is little thickness of the carpel here. But with this secondary thickening following pollination, the number of tannin cells increases within the region of carpellary fusion, as within the idioplast zone proper.

At maturity, the number of tannin idioplasts has increased enormously, while, also, the size of the elements is also much greater. The largest are upwards of .2 mm. in radial measurement, and over .1 mm. in diameter, though these measurements must be taken as indicative of the generally large size, rather than a specific statement of size, which varies with the race. I measured the tannin cells in a fruit of Deglet Noor.

Little further needs to be said by way of description of these cells. One may remark, however, that an examination of a full series of material suitably preserved, extending over the whole period of maturation from April to October, taken at intervals of two weeks, shows no evidence of the secretion of tannin within the parenchyma adjacent to the idioplasts indicating that these latter act merely as receivers of tannin or tannin-like material. If such is the case the evidence would be discoverable since it has been obtained elsewhere in the fruit and seed.

Intercellular Tannin.[15]—A peculiar condition is not infrequently to be observed, namely, the injection, as it would appear to be, of the intercellular spaces of the idioplasts and adjacent tissues with tannin.[16] I have been unable to fix upon the explanation of this though there is some evidence that it is due to bruising of the fruit. The masses of tissue showing this condition are irregular, but always associated with the idioplast layer. Aside from this, they are not at all constant in position. Wherever the surface of the fruit has been slightly damaged by pressure, as shown by brownish coloration, one may generally find more extensive injection of the intercellular spaces beneath than elsewhere. Where actual wounding and tearing of the tissues have intervened, there may be observed a still more pronounced appearance of the tannin in question. Whether however the injection of the intercellular spaces is due to an excretion or to rupture of the tannin-containing cells there is no evidence to decide. Vinson has observed that mature dates rupture on being placed in certain fluids, and this occurred in some of my own material, as well as some shrinkage at times. The unequal pressures that are set up would probably be sufficient to bring about the results described.

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[15] According to Winckler, extracellular tannin pockets occur, but I have been unable to see this paper.

[16] Howard (1906, p. 570); speaks of this condition, in the persimmon; I have recently confirmed his observation.

Similar injection of the intercellular spaces frequently occurs along the sutural tract, in contact with the idioplasts occurring there (f. 11).

*Mesocarp; endocarp.* The line of delimitation between the mesocarp and endocarp is not definite. It will, however, make a clear enough anatomical distinction to regard the toughish membrane which frequently adheres somewhat to the seed as the endocarp. This may vary in structure in being of from one to several cell-layers in thickness. The cells are somewhat elongated with square, oblique or irregular ends, occasionally sclerosed, and not infrequently, as Pond (1907) discovered by chemical methods, contain tannin which is similar to that found in the idioplasts.

The mesocarp embraces two zonal regions, the outer and inner. The outer mesocarp shows no peculiar anatomical changes in the later period of development. That sector of it which includes the tissues along the line of fusion contains a good many scattered idioplasts, similar in all respects to those of the periclinal zone, except that they are more or less elongated. The closer they lie to the seed, the greater their length; so great, indeed, that they are only with difficulty recognizable as of similar origin to the isodiametric idioplasts themselves.

The inner mesocarp may, for our present purpose, be described as a mixture of collapsed, chiefly elongated, thin-walled elements penetrated by a few displaced and distorted vascular strands. Here and there, isolated or in small groups, are very long cells rich in tannin. Sclerosed cells of similar form, having dimensions upward of 10 mm. in length and .2 mm. in diameter, their walls penetrated by simple oval or circular pores, and vaguely obliquely striated, are also to be found, more especially opposite each lateral angle of the seed, where a group readily visible to the naked eye, may occur. In transverse section, these stereids are circular or oval and sometimes contain tannin (f. 34).

Additional tannin is occasionally observable also throughout the mesocarp elements and bundles, in both hadrome and leptome. Frequently vessels filled with tannin have been seen. I am unable, however, to make a sufficiently detailed account of this phase of the matter to warrant any definite conclusions. This would require a more particular study of the vascular tissues than I have been able to give them.

There is some evidence that plastic tannin occurs in small amounts in the parenchyma of the mesocarp in certain regions. Copper acetate and ethyl nitrite material frequently displays a copper-red or orange coloration respectively, which blackens with iron salts. As maturity is approached, this behavior is not to be observed. Fresh material was not studied, and the facts just stated may be misleading, but it would seem to be the case that plastic tannin, similar to that to be described in the endosperm, occurs in small amounts in the mesocarp during development. The reaction upon which this conclusion is based is more marked in the sutural sector than elsewhere, and in the inner than outer mesocarp. In the latter, however, the amount, as previously stated, is exceedingly small at any time. For some time previous to ripening, as attested by Thornber, Vinson and myself, no tannin occurs in the major portion of the mesocarp. There is, therefore no evidence in this instance that tannin gives rise to any of the abundant sugar, one of the alternative views advanced (Bigelow, Gore, and Howard, 1906).

*Ovule.* Stage I. At the time of pollination there is tannin distributed throughout the whole of the ovule, as shown by a diffuse reaction. There is, however, a much greater quantity in the embryo-sac, inner integument, chalaza, raphe and pedicel (f. 2). The endocarpal pollen-tube guiding tissue shows a similar amount of tannin.

Regarding, for a moment, the anatomical facts recited above, it is difficult to avoid the conclusion that there is special nutritive activity connected with tannin both at the antipodal end of the embryo-sac and in the tapetum. In the Eleagnaceae (Servettaz, *l. c.*), while tannoid substances occur in the nucellar cells beneath the antipodal cells, and do not occur in the embryo-sac, it is argued that the antipodel cells play an active rôle. The conception of the physiological importance of these cells, in spite of their frequently small size and lack of histological peculiarities, is one to which we have become accustomed through the work of Westermaier, Goldfluss, Lloyd, and others,[17] but it still remains to determine in detail what their method of operation may be. Servettaz' suggestion should prove fruitful.

In the date, although the antipodal cells are small, their anatomical relations are peculiar. In the definitive embryo-sac, they are placed at the end of a slender pit, surrounded by elongated cells with thickened walls (f. 4). The thickening appears to be due to swelling, and this must, I think, be of significance since it is only the walls forming the antipodal pit which are swollen (f. 1, 3). Whether this is the same sort of thing observed by Servettaz (*l. c.,* p. 354) or not, I cannot say. He appears to regard the thickening and gelatinization of the antipodal cell walls observed by him as an accompaniment of disintegration. In the case before us, the swelling of the cell walls is not due to digestion, since their persistence (f. 5) shows the contrary. They appear, however, to be full of tannin, as they show a definite reaction at once on the addition of reagents.[18] A careful study of the development and nutrition of the embryo-sac previous to pollination will, I believe, show that they behave in some such way as suspected by Servettaz in, *e. g., Hippophae rhamnoides.* Unfortunately my material has not been suitable for this.

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[17] Coulter and Chamberlain, Morphology of Angiosperms.

I have been able, however, to follow the behavior of the antipodal cells after pollination. It appears that disintegration proceeds from the basal cell, operating successively on the adjacent and the innermost (f. 4, 4a). The disintegration is preceded by the reduction of the nucleolus, and the enlargement of those of the adjoining cells. The order of disintegration is the reverse of that followed in *Hippophae* and other Eleagnaceæ (Servettaz, *l. c.* p. 354). Whatever the explanation of the difference, their position at the end of the elongated cells which represent the end of the vascular supply to the embryo-sac, as in *Hippophae,* the presence and evident nutritive importance of tannin, the peculiar thickening of the contiguous cell walls, and their ultimate behavior, all offer positive evidence of their physiological importance.

It would similarly appear that the tapetum[19] is also important. At the early stage of development both tannin and starch occur in the inner integument and tapetum. This tissue does not break down—is not digested by the embryo-sac. The character of the tapetal cells indicates that its nutrients are transferred to the embryo-sac, following the decomposition of the thin nucellus.

Just before, or, at any rate, immediately following pollination, the endosperm begins a rapid chalazal growth, previous to the division of the secondary nucleus. In doing so, it digests the tissue at the base of the embryo-sac quite irregularly, and forms several cul-de-sacs which extend backward beyond the antipodal pit. This is, for some time, left projecting into the utriculum of the embryo-sac (f. 5).

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[18] This is not the secondary staining of the wall spoken of by Strasburger in his Practical Botany.

[19] Goldfluss, 1899.

Finally the tissue beneath it is undermined, and so a single antipodal cul-de-sac is formed, which continues its growth upward and toward the raphe. During this time the tissue about the cul-de-sac shows a reducing action on Fehling's solution. The cell walls are swollen, and stain deeply with Bismarck brown. Thus we may infer that active digestive processes are in progress; but we are not, of course, able to say whether the reaction is one to tannin alone, or to a reducing sugar, or both. The cul-de-sac is therefore a haustorium, for a prolonged period constantly encroaching upon the surrounding tissues. Its size increases as the seed enlarges till its dimensions are sufficient to make it a prominent feature of the mature seed (figs. 5, 6, 7, 10, 13, 12). It is important to notice in this connection that, resulting from the stimulus of the growing cul-de-sac, the surrounding chalazal tissue increasing concomitantly by cell division, its cells become radially disposed. These always contain tannin, the least densely filled cells being those next to the endosperm. A part, at any rate, of the food material supplied to the endosperm is a tannoid substance and this passes over into the endosperm chiefly at the cul-de-sac. As we shall see, a tannin is also present in the endosperm during some part of its development. That the chalazal cul-de-sac is the active center for the distribution of nutrients is indicated also by the circumstance that it is the center from which progress the physiological as well as anatomical changes which overtake the developing endosperm; and by the further fact that the remaining tissues contingent on the embryo-sac grow, *pari passu,* with the endosperm.

Why the endosperm digests the tissue touching it at one point and not at another is an interesting and important question, which has received little attention. The same question applies to the embryo-sac, during its development in many instances; and similarly to the developing embryo. I have elsewhere[20] shown that the embryo-sac may develop in a quite abnormal manner in this regard and behave much as a pollen tube, but in a more aimless fashion, so to speak.

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[20] Lloyd, 1902.

The facts observable with the microscope seem to force the conclusion that the mechanical conditions determine the behavior. Thus, in certain Rubiaceae, the embryo-sac extends forward along the micropyle, during the development of the single thick integument. If, as may happen, instead of remaining within normal bounds, it continues to grow until it extends beyond the exostome, it then pushes its way in any direction—the easiest mechanically. But if, instead of moving forwards, it develops backwards into the chalazal tissue, its path is indirect, and appears to be wholly indeterminate. In the date, however, the backward growth of the endosperm is direct and determinate. To be sure, it is pointed in the right direction to begin with. Though it digests the nucellus—a very thin layer—and to this extent moves forward, it is resisted by the integumental tapetum. So far the same phenomenon is seen in other plants (*Elaeagnus,* Servettaz, *l. c.* Cucurbitaceae, Kirkwood, 1904) but to a more marked degree. This resistance may, probably, be explained by a chemical response, or by a resistant layer, say a cuticle, as observed in *Tricyrtis* by Ikeda (*l. c.*). But how shall we explain the direction of the antipodal haustorium, unless we assume, as a working hypothesis, that the response is chemotactic and that, therefore, many facts about the embryo-sac are to be explained in the same way that we explain the behavior of the pollen tube! To assume a local enzymatic activity would be, I think, to assume too much, though this also is not impossible.

Later stages (the young seed). During the whole period of development until maturity the raphe and integuments contain iron-blue tannin. For some time—no exact period can be determined—the reaction in the integuments is diffuse, as it is in the bulk of the raphe, in which, however, are groups of cells showing a superior content. The appearance of these cells suggests that the tannin in them is aplastic, while their position, which is removed some distance from the endosperm, also indicates this to be the case. Similarly, as stage III is approached, there is evidence below the level of the embryo, that the tannin is accumulating in permanent form in some cells. At this time (f. 14) the endosperm cells in contact with the raphe and the integument contain more tannin than the inner ones, and it would seem that the integuments serve in some measure to distribute the tannin. The markedly greater activity in this regard of the chalazal tissue has already been noted.

As maturity is approached, many of the cells of the outer integument lose their tannin. These are chiefly the epidermis and an irregular zone of underlying cells, in all of which not the least trace of tannin is to be found (f. 31). The cells of the inner integument are all tanniferous. What becomes of the tannin in the cells which definitively do not contain it is not easy to say. There is no evidence that it is removed to adjoining cells; on the other hand, there is none that it is transformed, beyond the circumstance that there is a considerable sclerosis of the epidermal cells. It has been noted that of cells of similar origin, apparently, in the endocarp, some become sclerosed and do not contain tannin (or occasionally, very little), while others remain thin walled, and are tanniferous. This lends some probability to the view that the tannin is used in physiological changes in the individual cell. If, however, the removal of tannin from the epidermis and underlying cells is to be explained by lateral movement into those cells which are finally tanniferous, this happens long before the period of ripening, and is not the rapid segregation described by Howard (*l. c.*) in the pericarp of the persimmon.

After ripening, the cells of the integument and raphe, with the exception noted, are heavily loaded with insoluble aplastic tannin. To this the deep brown color of the seed is due.

To sum up briefly, it appears that the tissues of the ovule, setting aside the endosperm for the present, are, during the period of growth, supplied with plastic tannin which is passed over to the developing endosperm. During the same period, tannin cells containing aplastic tannin appear, few at first in the raphe, but at length throughout both raphe and integuments. During the latter part of the interval between stages III and IV, tannin disappears from the outer part of the outer integument. All the tannin finally becomes insoluble; it is found to be so in the ripe fruit.

There is evidence that during development tannin moves from the integuments and raphe into the endosperm, as well as from the chalaza, where the activity is especially evident (f. 27a).

*Endosperm.* Stage I. A strong tannin reaction is seen in the embryo-sac. Just at the time of pollination the endosperm nucleus and its protoplasm become blackened by iron following copper. The same evidence is had by the use of ethyl nitrite, which gives a brown reaction. Alcohol material gives a less pronounced, but readily observable reaction. For a period of eight weeks, or somewhat longer, the endosperm retains a syncytial character, and remains as a thin protoplasmic lining. All the material collected during this period (Rhars and Deglet Noor— in alternate weeks) shows that during the whole of this time, the endosperm is always replete with tannin. Copper acetate and ethyl nitrite material invariably show also a thick precipitation membrane lining the endosperm utriculum, from which it must be argued that the fluid within was rich in soluble tannin. It is also to be inferred from the facts of distribution, that the tannin is diffusible, and this is supported by a study of the endosperm in a later stage.

Stage II. From this time on the endosperm develops centripetally (f. 10), but this more rapidly in the chalazal cul-de-sac than at the micropylar end. During the periods of growth between stages I and III, there is a general distortion, producing a displacement of the embryo, so that this comes to lie definitely in the familiar midway position. (f. 12). The filling of the utriculum occupies about four weeks, in which time the seed attains a length of 9 mm. with a diameter of 3 mm. (Deglet Noor). The lack of any pronounced tannin reaction (more marked in the peripheral cells than elsewhere) at this time, indicates that there has been a substitution of one material for another. As a matter of fact for the first time there is to be noted a general distribution of oil in small droplets throughout the endosperm, but whether there is any causal relation to be traced is not to be decided off-hand.

Stage III. Very soon the thickening of the cell walls, to form reserve cellulose, begins, (f. 26 a). Concomitantly, there is *a reappearance of tannin,* so that, in material two weeks older, (3 months old) there is tannin throughout[21] the endosperm in large amount (f. 27). The amount is however much greater in the central portion, namely in those cells, strictly speaking, which are undergoing secondary thickening of the cell walls, looking toward the definitive condition of the seed. The reaction at this time is very pronounced, and the tannin-bearing portion of the endosperm forms the most conspicuous feature of the seed macroscopically (f. 27).

As I had some doubt of the facts here presented, another lot of material was obtained for me by Professor R. H. Forbes, and treated by Dr. A. E. Vinson with ethyl nitrite. The material, Deglet Noor, included material pollinated March 9, and fixed June 9, 1910. Upon examining this, the whole of the endosperm and the embryo were perfectly white, a quite unexpected result. The area (shown by stippling in f. 28) which should have been deeply stained, was a more opaque white than the surrounding zone. But upon the addition of ferric chlorid, a prompt reaction followed, in quite the expected manner. Upon prolonged treatment with very dilute ferric chloride, the central part of the endosperm was stained dense blue-black, with the outer zone obviously less so. Re-exposure to vapor of nitrous ether produced the reaction also, from which we may infer that the period of original exposure was insufficiently prolonged.

During another two weeks the seed grows rapidly, attaining a transverse diameter of 7 mm. (f. 29), and the tannin tissue has extended toward the periphery, but is less pronounced in its response to reagents. After this, the density of the reaction is quickly reduced, but extends quite to the periphery. I have found a relatively small amount of tannin present even as late as October 8, in Deglet Noor material pollinated on March 28. Frequently the disappearance of the tannin is even in rate throughout the whole mass of the endosperm, but one more often observes that it is at different rates in different parts. In some instances (f. 28, 30), and these are particularly instructive, the tannin remains longer in certain sectors, having their vertices at the line of fusion marking the obliteration of the endosperm utriculum, than in others. The wedge-shaped areas (f. 28, 30) thus made conspicuous, reach quite to the outermost layer of the endosperm, and their superior tannin content is quite evident to the unaided eye. In other cases, similar but irregular areas occur, sometimes centrally placed and showing a radial disposition, or it may be less definite relations. On the whole, the tannin is retained rather longer in the general region of the raphe than elsewhere, thus suggesting a movement of the material concerned from this part of the ovule into the endosperm. The last evidences of tannin are to be seen in the outermost, peripheral endosperm cells, which are the last to take on their definitive character. These are at this time passing from an obliquely distorted condition, due to shearing pressures exerted by the endosperm on the one side and by the integuments on the other. With further growth they finally attain a radial disposition, and the tannin content entirely disappears, save in isolated positions. The mature endosperm shows very little tannin, and this is confined to irregular sulci, caused by uneven rates of growth and the consequent tangential crushing of peripheral portions of endosperm which are unable to thicken their walls, and to adjust themselves finally to the surrounding tissues. The minute quantity of tannin found by Pond (1906, p. 74) is probably to be explained by this circumstance, though a partial explanation may be had in the incomplete eradication of tannin elsewhere.

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[21] See also p. 134.

From the circumstance that there are no special tannin cells in the endosperm at maturity, we must conclude either that it disappears as the results of chemical change or that it migrates into the surrounding integuments. The facts described above do not consist, I believe, with the latter alternative, but are quite applicable to the former.

The appearance of tannin simultaneously with the beginning of secondary thickening of the endosperm cell walls has a good deal about it to warrant the belief that the tannin plays some rôle during the formation of reserve cellulose. The reaction is not in the cell lumen, but definitely in the cell wall, and, in the more deeply reacting cells, in the primary membranes. This appears not to result from displacement of tannin[22] as the reaction appears at first in the cell wall. In copper acetate material, in which we may imagine displacement of tannin from the lumen into the wall to have occurred, the tannin reactions in other tissues (e. g. the raphe) are in the lumina. Ethyl nitrite material gave identical results in the clearest and most convincing fashion. The endosperm, as stated elsewhere, did not show coloration, but, upon the application of iron acetate, gave a prompt, iron-blue reaction in the cell-walls, but not in the lumina. In the integuments and raphe, a similar reaction was given in the lumina of the tannin cells.

This parallelism between the distribution of tannin and the thickening of the cell walls, and the ultimate disappearance of the tannin when the endosperm is fully developed, indicate an analogy between the tannin in question and other nutrients in transitory form. For this view speaks the fact that the tannin in the endosperm is throughout diffusible, since, in contrast to the tannin in specialized cells, it is found permeating the cell walls. This conclusion is justified from another point of view. Strecker, according to J. Reynolds Green[23] has shown that a "tannin" is a glucoside, yielding gallic acid and glucose by hydrolysis. The fermentation of infusions of certain galls, yielding, with the disappearance of tannin, gallic and ellagic acids, (also pointed out in Green's work) taken together with the hydrolysis just mentioned, indicate that the comparison of the plastic tannin in the date endosperm with transitory starch is substantiated. Confirmatory evidence is had further in the fact that fungi can assimilate tannin, among other aromatic substances,[24] though anything beyond a very low food value is questioned. Such tannin as is here described for the date may, however, not be a food of direct availability, but may be a link in a chain of metastates, or may afford energy in a more direct way, as by oxidation.

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[22] See above, p. 132.

[23] The Soluble Ferments and Fermentation, p. 160. See also Dekker, 1906.

*The Embryo.* An account of the occurrence of tannins in the embryo requires only the general statement that during the earlier stages of development, from pollination on, the embryo, together with the embryo-sac structures, and after fertilization, with the developing endosperm, is richly supplied with tannin. A pronounced general reaction is exhibited by the embryo as late as thirteen weeks after pollination, (Deglet Noor,) when the embryo measures .50 by .42 mm. When full sized, the embryo measures 1.1 mm. by 2.1 mm., an increase which is accomplished in a period (13 weeks) equal to that required to reach the smaller dimensions. During this second period the tannin content of the embryo becomes less and less marked. Nevertheless, until a short time before maturation, (f. 18) a distinct reaction is discoverable, and, though it is diffuse throughout the tissues of the embryo, there is evident a more marked amount of tannin (1) beneath the epidermis of the cotyledon, (2) along the vascular tracts, (3) in the plumule and the tissue beneath it, and (4) in the active zone of cells between the hypocotyl and radicle, which, of course, are both very short and not readily distinguishable. These peculiarities of distribution are clearly connected with the physiological function of the tannin. At maturity, and during the resting condition tannin is quite absent, as stated by Sachs. There are, so far as I have observed, no special tannin cells in the embryo during the embryonic or the resting period. In early germination there is according to Sachs' account, a reappearance of tannin, which soon becomes localized. When the extra-seminal portion of the seedling has reached a length of a few cm. the constricted zone of the cotyledon, connecting it with the haustorium, shows a definite tannin reaction. I studied fresh material of the ordinary dates of commerce. Iron chlorid shows, in common with methyl blue, (1) a general reaction throughout all the tissue of the isthmus and (2) a considerable number of special tanniferous cells in the parenchyma in the same region. These react as strongly as the idioplasts. The diameter of the neck is one-half that of the cotyledon just outside the seed, so that the areas of the cross-sections are as 1:4. The reduction of the area does not, however, affect the transporting capacity of this part of the cotyledon, since there is no constriction of the vascular tissues. It would appear therefore that these tannin cells are not to be explained by any nutritive relation. It is aplastic tannin, and, as Sachs believed, plays no active rôle. In the further nutritive phenomena seen in the growth of the haustorium or in the digestion of the endosperm, there is no evidence of tannin. In the cortex of the seedling, chiefly near the growing end, in the region therefore of the hypocotyl, there is observable a slight tannin reaction, but the facts are such as not to lend themselves readily to any explanation. At the close of germination, there are tannin cells distributed throughout the cotyledon, but chiefly in the ventral moiety and in the parenchyma immediately adjacent to the vascular strands. The ventral epidermis contains also a goodly number of tannin cells. The cotyledon is of course moribund at this stage.

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[24] Nägeli and Reinke, quoted by Pfeffer, Physiology of Plants, p. 492. M. T. Cook has shown recently that tannin is a poison. It is, of course, obvious that different substances may be meant.

In the first foliage leaf a similar behavior is to be seen save that the tannin appears more especially in the parenchyma of the dorsal moiety. In the second foliage leaf, before it has yet emerged from the encasing, cylindrical first leaf, the tannin appears at first in the parenchyma, in scattered cells lying in the middle zone. There is no diffuse reaction in any case, the tannin being completely confined to the cells in which the secretion at first takes place. The genesis of the tannin may be observed very exactly in the second foliage leaf, at the age indicated. It first appears as minute granules which become larger. These finally coalesce to form a continuous layer about the cell, or, at length, a mass with vacuoles. There is no evidence of a transitory tannin, so that it must be regarded, in the absence of definite evidence to the contrary, as aplastic.

### OIL.

No oil appears in the carpel at any time.

In the endosperm oil appears for the first time after about the seventh week following pollination, before the endosperm utriculum is obliterated (f. 8-10). There is a gradual increase, in the form of minute droplets, till about the tenth week, about the time, approximately, when the thickening of the cell walls begins in the endosperm. As already pointed out, this is the time when the tannin appears in connection with the thickening walls. For a short period (f. 26, 26a) at about the time of this phase of development, the oil content is reduced, during which time the seed reaches its definitive form. The oil content then increases again till, in the mature seed, the endosperm cells contain two carbohydrate foods, reserve cellulose and oil. According to du Sablon (1897) the oil constitutes 7-9% of the whole endosperm in the resting seed. I find no evidence of tannin within the resting endosperm save in certain cases due to irregularity of development already noted.

The disappearance of oil and the beginning of cellulose accumulation are without much difficulty explained on the same grounds upon which we may also explain the accumulation of oil as the reserve cellulose approaches a definitive condition. The relation of tannin to these is more obscure, but there is sufficient evidence, already advanced, to regard it as related in some way to the changes which go on during the thickening of the cell walls.

## NUTRITIVE RELATIONS OF THE EMBRYO AND ENDOSPERM.

The nutritive relations existing between the germinating seedling and the endosperm have been so frequently investigated that the date has become classic. Under the above caption, it is my purpose, so far as possible, to complete the account by detailing the corresponding relations during the embryonic period. Especial interest attaches to this phase because the growing embryo opposes itself to a growing endosperm, while during germination, the latter is wholly passive.[25] This opposition of two growing and anatomically independent bodies must result in adjustments which may be mechanical merely; or they may be chemical with resulting secondary mechanical adjustments. There must also be a zone where the counter-influences meet each other, which may be termed the tension zone.

### Periods I and II.

During the first and second periods, as I have already pointed out, the growth of the embryo is slow. A week after pollination it measures about 25 microns in diameter, and attains at stage III a diameter of only 60-75 microns. It is evident that, in view of the loose anatomical character of the endosperm, and the slow increase of the linear dimensions of the embryo, there is but little mechanical readjustment involved. During the first period, I have found no optical evidence of digestive activity peculiar to the embryo, although, of course, this may obtain. This is apparent from the fact that, at 11 weeks, all the endosperm cells near the embryo are intact (f. 14a), and show no evidence of digestive action in the cell walls. It seems probable therefore, that the embryo and endosperm act as a physiological unit so far as nutrition is concerned. At any rate, the reactions appear to be quite the same in both, and show, e. g., that tannin is abundant in them throughout the first period. Toward the close of the second period, although there is pressure upon the endosperm surrounding the embryo, there is still no digestion of the cell walls to be noted. There is therefore no breaking down of tissue. During the early part of this period, however, there is to be seen the independent action of the embryo in the unequal distribution of oil in the surrounding endosperm (f. 14). At this moment the accumulation of oil throughout the latter is marked, but much less so in a spherical mass surrounding the embryo. This sphere measures fully 250 microns, so that the embryo exerts an influence to a distance of some 100 microns, more or less, from it. In addition to the small amount of oil in this region, I have noted that my preparations of material of this age all show an accumulation of tannin in the form of droplets (f. 26, 26a). I have not seen it in the same or corresponding region in this form at any subsequent time. The preparations in question were fixed in copper acetate, treated with ferric chlorid and with alkanet, before and after absolute alcohol and chloroform, so that there can be little doubt of the accuracy of the observation. This appearance of tannin is synchronous with the beginning of reserve cellulose formation, when, as already stated, tannin appears in that portion of the endosperm where the thickening of the walls occurs. It may therefore very well be that this tannin is concerned in the nutrition of the embryo, but my material was not sufficient to settle the question and it must in consequence be left open.

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[25] Pond, 1906.

To complete the evidence that oil is a nutrient for the embryo at this time it should be noted that it occurs plentifully in the embryo as well as in the endosperm. The appearance of the oil in the embryo differs from that in the endosperm. In this, when the oil first appears, it occurs in extremely minute droplets, which collect in a zone about the nucleus. This is especially well shown by the pavement endosperm cells lining the cavity (f. 9). In the embryo, however, this relation is not to be seen. Here the oil occurs anywhere in the cytoplasm, as depicted by Sachs in the epithelium of the growing haustorium.

How the distribution of oil at this time is to be understood is not immediately clear. There appear to be two alternatives. The comparative absence of oil in the vicinity of the embryo may be due to the immediate absorption by the embryo of most of the material which would, in this region, be turned into oil; or to the movement of the oil, after it has been laid down, toward the embryo. In the latter case, we may assume a lipase to account for digestion, or we may assume that the fatty material moves in the form of minute globules, as Sachs believed. The occurrence of droplets of oil between the embryo and the endosperm was regarded by Sachs as evidence that during germination the immediate entrance of this substance into the embryo is accomplished without change of its molecule. I myself have observed the occurrence of large droplets of oil in the same situation during both the embryonic period and germination. Figure 15 is an example of the former, which, however, is explainable otherwise. Assuming that the oil actually occurs here normally, it seems probable that it is due to the accumulation of small droplets from the disintegration of the cells in which it occurs. (zone *a*, f. 15). This would readily be brought about if the digestion of the oil at this time did not proceed as rapidly as the breaking down of the cell walls.

The large masses of oil in the space between the haustorium and the compressed endosperm during germination on the other hand, I believe to be purely accidental, and is due to the transposition of the oil by the knife-blade during sectioning. There is, furthermore, convincing evidence that during the major part of the third period of embryogeny, as well as during germination, that there is an actual digestion of the oil, resulting in water-soluble substances, and it is therefore entirely probable that the same is true of the still earlier periods during which oil plays a part in the nutrition of the embryo.

### Period III.

The final period of development is of peculiar interest in that it leads to the articulation of the two distinct physiological periods, that immediately before and that following, germination. Of much information regarding the latter we are already in possession, chiefly from the work of Newcombe and of Pond, following that of Sachs, Reiss, du Sablon, and others.

The period in question involves a marked mechanical adjustment of the embryo and endosperm. At first the embryo is spherical in form (f. 14) becoming, at 13 weeks (f. 16) broadly ovate-conical, with the base against the micropyle. Scarcely any change in shape is now to be noted for two weeks, after which, however, the growth is rapid. Within another month, the definitive form and almost full size are attained. (f.18). This, especially, is the period of mechanical adjustment, for it is now that the endosperm is also growing rapidly, and that the secondary thickening of the cell walls, to form the reserve cellulose, takes place. But during this time approximately, the radial growth of the endosperm is relatively much more rapid than that of the embryo, and there arises as a result a cavity in the endosperm, opposite the cotyledonary end of the embryo, which is at length nearly obliterated (f. 15-19). For the purpose of enabling the reader to follow the changes which ensue during this period, I have chosen six stages of development, and represented these in figures 14a-19, inclusive, to which the following descriptive notes may be applied.

Figure 14a.—Zone *a*. Cells show no disintegration, but are large, with a relatively small amount of protoplasm, and reduced oil content. No blue reaction in the cell-walls with iodine.—Zone *a* becomes differentiated into zones *a* and *a'*.—Zone *b*. Small endosperm cells with divisions taking place approximately parallel to the integument. This characterizes also the peripheral endosperm, beneath the inner integument.—Zone *b'*. Thin-walled endosperm cells, larger than in zone *b*.

Figure 15.—Zone *a*. Cells with collapsed walls, empty or with minute protoplasmic content and a droplet of oil. *The walls react blue to iodine.* In the space between *a* and the embryo, oil accumulates.—Zone *a'*. Uncollapsed cells within the digestion zone. The outermost show no change. The innermost show a more or less disintegrated

condition in the protoplasm. *The walls react blue to iodine.* The small quantity of oil becomes more evident in the inner cells as droplets removed from the protoplasm.—Zone *b-b'*. Thin-walled endosperm cells in which cell divisions occur, more especially in the layer of cells between *b* and *b'*. Tension zone, contributing cells to zone *a'* and to zone *c*.—Zone *c*. Endosperm in which the thickening of the cell walls has progressed. The limit between zone *b* and zone *c* is not a sharp one. (Zone *c'* is not visible, but has begun in the interior of the endosperm. cf. fig. 26a.)—Zone *b'* now becomes compressed between the advancing thickening endosperm, zone *c*.

Figs. 16, 17 and 18.—Zone *a*. Cell walls entirely collapsed, reacting blue with iodine. All the contents except minute droplets of oil have entirely disappeared.—Zone *a'*. Cell walls not collapsed, reacting blue with iodine. The cells nearer the embryo have no other content that each a single large droplet of oil. The outermost have more or less protein content.—Zone *b*. Unaltered endosperm cells, the inner of which are subject to digestion. The outermost cells of this layer are represented by zone *b'*.—The tension zone becomes narrowed down to zone *b'*, of cells undergoing division, and shearing between the growing endosperm and zone *b*.—Zone *c*. Endosperm with thickening cell walls, giving a tannin reaction only in the lumen. Zone *c'*. Definitively thickened endosperm cell walls. Deep tannin reaction in the cel-walls in the younger stages but disappearing in the older ones.—The zones now become compressed and the distinctions less marked or nearly obliterated.

Figure 19.—Zone *a*. Crushed and compressed cells reacting blue walls and contents. This zone is separable into two zones by the successful application of iodine and sulfuric acid. This treatment produces a deep blue reaction which quickly fades away, and causes swelling and dissolution. These proceed differently however in the two layers a and *a'* figure 20. The compressed cell walls of layer *a* swell and dissolve as a whole; those of *a'* first expand and assume their original form previous to swelling, indicating that they are not so far disorganized by digestion as those of *a*. The compressed cells contain numerous minute oil droplets; the less compressed (*a'*, f. 20) large droplets.—Zone *b'*. Tension zone. The cells are partially thickened, less toward the embryo, more toward the outside. They are secondarily divided by transverse walls. They contain protoplasm and oil, the latter in large drops, the protoplasm more or less disorganized.—Zone *c*. Definitively thickened and characteristic endosperm cells.

It may here be noted that the distribution of oil in the compressed zones is not as figured by Sachs (his figure 4, pl. 9, 1862), who represented large drops as occurring anywhere within the compressed layer. The treatment with sulfuric acid demonstrates this very beautifully, as the droplets are displayed in their relative positions as the wall material swells. This is true of the whole period of germination.

We are now in a position to present in summary form the changes which occur.

*Zone of Digestion.* At first a spherical mass of intact cells (*a*, figure 14a) this becomes differentiated into two zones (a, a′, figure 15) due to the collapsing of the walls in zone a accompanied by the digestion of their contents oil and protein. We have therefore to note (1) the cause for the collapsing of the cells which is found in the digestion of the primary walls; (2) the disappearance of the contents, due to the digestion of the oil and protein.

*Digestion of primary cell walls.* Sachs (1862) believed that the primary membrane is not digested during germination, but that the growing haustorium pushes the exhausted and crushed cells before it. Reiss (1889) extended Sachs' observation of the endosperm of *Chamaerops humilis.* I have found no evidence to the contrary after a stage of germination roughly indicated by an embryo length of 2 cm., at which stage the haustorium has attained the form of a sphere (f. 24), so that there exists no lack of harmony between my results on germination and those of Sachs and Reiss, which have been generally accepted.

Newcombe (1899) extracted a ferment capable of digesting the *whole* of the endosperm cell wall. This result is of interest in that there is yet advanced no optical evidence that the primary membrane is actually digested in the date. Newcombe's results appear to indicate that there are two ferments extractable together,[26] one capable of acting on the primary wall, the other on the reserve cellulose. The former may be present during germination in such small amounts that the result of its action on the primary wall is inappreciable to the eye, but that, during the prolonged period of experimentation made use of by Newcombe, it had time to act. Probability is lent to this interpretation by the work of Green.

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[26] The contrary view that a single ferment isolated by Newcombe is capable of digesting both primary walls and the reserve cellulose is negatived by the positive evidence that such digestion does not take place during germination; though the logical possibility remains that it takes too long or that it is inhibited in some way.

Green (1899, p. 97) in 1887 studied the progress of digestion in the germinating seedling of *Livistona humilis,* which is quite similar structurally to the date. After two months germination at which time the endosperm was about half absorbed, Green found that the "inner zone"[27] of the digested portion of the endosperm gave a blue reaction with iodine, but failed to react with chlor-zinc-iodine. Green concluded that under the action of a cytatic ferment the cell wall (primary membrane) was changed. This form of digestion in the primary membrane Sachs did not see in the date, and, as far as microchemical methods may tell us, it appears that he was right. During the embryonic period, however, it is otherwise. My observations show that soon after a stage represented by figures 14a, 26, when the adjustments between the embryo and the cell wall of the endosperm are purely mechanical, a substance is secreted, presumably by the embryo, which acts upon the nearest cells, attacking the primary membrane and changing it into an amyloid, as I may call it for the present. The reaction indicative of this conclusion is to be seen clearly at a stage represented by figure 15, in which a column of cells (*a,* in the figure) opposite the apex of the embryo all show a distinct and characteristic blue coloring. The walls themselves in zone *a* appear wavy and more or less crumpled by lateral pressure of the cells of zone *a′*, and show every appearance of undergoing some sort of change. The blue reaction is shown also by the walls of the uncrushed cells (a′, f. 15) flanking the column of crushed cells (a, f. 15). This behavior in the presence of iodine indicates clearly that the membranes are undergoing a chemical change, hydrolytic in nature, similar to that observed by Green during germination in *Livistona.*

That this digestion is such in fact is shown also by the behavior of the crushed cell walls (a, f. 20) nearer the embryo as compared with those in a layer further removed (a′, f. 20). The former are directly dissolved by sulfuric acid while the latter spread out and take their normal form before dissolution. The exceedingly tenuous character of the crushed walls next the embryo, as compared with the thicker condition further away also points to the same conclusion. It may finally be noted that the earliest mechanical disruption of cells is not due to the pressure of the embryo upon the contiguous endosperm cells, but of the cells of zone *a'* upon those of *a* (f. 15). It takes place previous to the secondary thickening of the endosperm walls by the deposition of reserve cellulose. As the embryo grows, the amount of tissue thus affected increases markedly (f. 16, 17) until a time of maximum activity is reached, which happens about eighteen weeks after pollination, at a stage represented approximately by figure 18. The reaction obtained at this time indicates that zone *a'* (f. 18) may reach quite near to the outer limit of zone b. My preparations show further that the blue reaction is not always confined to the cell wall, but may frequently fill the lumen, indicating the presence of a soluble or colloidal substance here.[28] On approach to maturity, the relative thickness of the layer of cells affected becomes reduced, and at the time of ripening, occupies the zone *a* shown in figure 19. I have verified this by control with commercial dates, which give the same reaction.

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[27] I understand this to mean that next the haustorium.

During the early stages of germination I have found only a partial reaction and this is confined to the endosperm in contact with the older part of the growing haustorium (f. 24, 25). In a stage corresponding to figure 25, the blue reaction appeared along the flanks and around the end of the cotyledon, but in the latter position it was very thin and discontinuous. In a somewhat older condition, as in figure 24, the reaction was to be seen only along the sides of the haustorium, corresponding to the extent of the sides of the cotyledon seen in figure 25. It seems probable therefore that the change of the primary membranes into an amyloid is chiefly an embryonic phenomenon, and ceases during or perhaps previous to, the early stages of germination. It is a fact of very great interest however that this form of digestion, which in *Livistona* takes place during the course of germination, occurs in the date only during the embryonic period. I therefore conclude that there is secreted by the growing embryo during the period from the age of about 10 weeks till maturity an enzyme, probably a cytase, which causes the hydrolysis of the primary walls of the developing endosperm in the region of the embryo. It is to be further inferred that this digestion leads to the formation of a sugar, in which form it is absorbed by the embryo.

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[28] I have found some evidence that a small amount of starch in the form of minute granules occurs. These are scattered between the crushed cell walls. The amount seen however is very small, and I have not always been certain of its presence. This uncertainty may be due to its disappearance and reappearance.

*Digestion of oil and protein*—(a) During embryogeny.—The separation of the zone of digestion into two sub-zones, a and a′ in the figures, of collapsed cells within, next the embryo, and uncollapsed cells further out enables us to describe the course of events relative to oil and protein with reference to the mechanical condition of the cell walls.

At the beginning of the period the zone (*a,* f. 14a) is undifferentiated within itself. The cells contain only small amounts of oil (f. 14). Before the digestion of the cell walls, as already described, sets in, the amount of oil increases. The digestion of a column of cells in front of the embryo (*a,* f. 15) is accompanied by the disappearance of the protoplasm and the isolation of the oil so that small droplets are seen within the strand of crushed and empty cells. Assuming for the moment that there is an actual digestion of this oil, it must be slow, since there collect large droplets in the space next the embryo. That the breaking down of the cells at this time should proceed more rapidly than the undoubtedly slow digestion of oil may be understood by the fact that the volume of these cells is relatively much greater than if their compression were due merely to the pressure exerted by the embryo. In point of fact, this pressure is exerted by the adjacent undigested cells in the direction indicated by the arrow points in the figure. Now begins the rapid growth of the embryo, accompanied by the compression of the cells adjacent to its surface (f. 16), but the growth of the whole endosperm in radial direction, as indicated by the arrow point at the bottom of the figure, results in the formation of a pit in front of the cotyledon. This is obliterated only with complete maturation (f. 19). The crushed layer of cells lines this pit, their crowding, which does not amount to compression, still caused by the adjacent tissues. The later progress of events consists in the amplification of these relations till a stage represented by figure 18, is reached, followed by a reduction during maturation.

Observation during the whole of the period shows that the distribution of protein and oil in these tissues may be briefly summarized.

The outermost of the uncollapsed cells (zone *a′*) contain both their oil and protoplasm in practically an unchanged condition. Passing toward the embryo, the protoplasm shows evidence of disintegration and obvious reduction in amount till the inner layer of uncollapsed cells is reached, where only the oil remains usually as a single drop in each cell. Within the crushed layer, these oil drops are quickly reduced in size (f. 18a and 20).

(b) During germination.—The zones[29] of digestion surrounding the growing haustorium are as follows, beginning at the plane of contact with the embryo (f. 24; text figure B). The thickness of the zones depends upon the position relative to the haustorium, being thickest opposite the rapidly advancing edges:

*a.* Crushed cells derived from embryonic period, reacting blue with iodine. This zone is discontinuous and is derived from the resting condition.

*b.* A zone of compressed cells, of primary membranes only, about 15 microns thick. The oil is in minute droplets. (Sachs' layer 1).

*c.* A zone in which the oil is in a large single drop in each cell; the protoplasm has disappeared. The reserve cellulose is less in amount proceeding toward the embryo and the cells are more compressed (text figure b). (Inner part of Sachs' middle layer.)

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[29] The reader will not infer that these zones are sharply delimited, any more so than the others which I have described. Nevertheless, they represent an objective reality.

*d.* A zone from which the protoplasm is disappearing, and in consequence, the oil becomes agglomerated. The reserve cellulose begins to undergo change. This zone takes up Bismarck Brown more readily than elsewhere. (Outer part of Sachs' middle zone.)

*e.* Unchanged endosperm. (Sachs' corresponding layer.)

This somewhat sharper analysis than that of Sachs has for its purpose to show that the agglomeration of the oil is coincident with the disappearance of the protoplasm, and that the disappearance of oil begins when the cell-walls are reduced to the primary membranes. This harmonizes with du Sablon's conclusion that the amount of oil in the undigested endosperm remains the same (7-9%) during germination, and that "on peut donc dire que la matiére grasse de l'albumen n'est absorbée par le cotyledon qu'au fur et à mesure de la digestion des parois mêmes des cellules de l'albumen" (1899, p. 396). It may be observed however that the relative amount of oil in zone *c* is greater than elsewhere, because of the reduction in the amount of both the protein and reserve cellulose. The comparison of the behavior of oil and protein during embryogeny and during germination shows further that the facts are identical, while the cessation of growth in the endosperm and the presence of reserve cellulose remove one condition, the presence of the tension zone, and impose another, the necessity of digesting the reserve cellulose, a different material from any attacked by the embryo previous to the resting stage.

The conclusion seems obvious, that during the embryonic period the oil is digested and, before absorption by the embryo, changed into water-soluble substances. This conclusion must be extended to the period of germination. Sachs believed to the contrary, for the reason, before stated, that oil occurs in large quantities in the compressed layer and in the space between this and the epithelium of the haustorium, and because the immediately underlying layer of epithelium also contains oil-droplets[30] (1862, p. 250). The presence of only a small amount of oil in the embryo was noted by du Sablon, but he observed also notable quantities of sugar (*l. c.* p. 398).

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[30] Reed (1904) in his studies of the cytology of the epithelium of the date appears to have overlooked the important modifications in the appearance of the cytoplasm, and possibly of the nucleus, which

I have already explained the presence of oil in the space between the embryo and endosperm as due to accident in the cutting of freehand sections. It would, I think, be apparent to anyone at a glance that this explanation is the true one. The presence of oil in the embryo is explainable on the ground that the materials derived from the endosperm which are not immediately used by the growing embryo are reformed into oil and starch. This explanation was applied by Sachs to starch. It is evident that the presence of a "notable proportion of sugar" was regarded by du Sablon as the part equivalent of the oil. My own contention is that the oil found in the embryo is derived from the water-soluble materials, derived from digestion of oil, and which, upon entrance into the epithelium of the embryo, are not removed by use, either there or elsewhere. In support of this view the following additional evidence is advanced:

In a germinating seedling, the extra-seminal portion of which was two millimeters long (figure 25) the digestion of oil in the endosperm *in front of* the end of the cotyledon, which alone at this stage is haustorial in nature, was proceeding. There was, however, no trace of oil to be found in the epithelium of the cotyledon in this region, but only in that flanking the cotyledon, opposite endosperm cells in which no digestion is going on. The distribution of oil is shown in figure 25 by the stippling. In the same general region, but in the parenchyma beneath the epithelium there is starch to be found. In a somewhat older seedling (f. 24) there was no oil to be found in the epithelium at all, although

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must ensue upon the accumulation and reduction of oil, which doubtless occur during relatively short periods of time. In examining living material, Reed further speaks of numerous large granules all of which react to protein reagents, but does not indicate that other reagents were employed. The subject of Reed's study is admittedly peculiarly difficult, and for this reason it is all the more important that the appearances of the cells in question should be explained only after taking all the substances occurring in them into consideration.

the region of digestion had by this time spread along the flanks of the cotyledon. In two seedlings in which the first foliage leaf was 3.5 cm. long, in which therefore the haustorium was quite large and nearly fully developed, I found the oil very unevenly distributed. In most of the epithelium cells there was none. A comparatively few cells in isolated groups showed a rich oil content. In no case was there any oil in the parenchyma below. In a similar seedling after a week's exposure to ethyl nitrite, there was a very large oil content, the epithelial cells being quite replete,[31] so that one could hardly observe the remaining contents. Scarcely a cell varied from this condition. Similar results were obtained in a younger stage when the first foliage leaf was just emerging.

The conclusion is therefore drawn that the disappearance of oil during the development of the embryo and during germination is due to the same cause, namely, digestion. This digestion results in the formation of water-soluble substances, and not of an emulsion. The reappearance of oil in the embryo is due to its reformation from these substances in consequence of the failure of the embryo to consume them as rapidly as formed.

The particular behavior of the protein I have not especially considered, beyond to note the time of its disappearance from the cell relative to that of the oil. Sachs believed that "albumen" enters the embryo "as such." It is apparent that this material must undergo proteolysis if we accept evidence of the same character as that advanced with regard to oil and cellulose.

*The Tension Zone.*—The limits of the various zones are not sharp, so that, in making diagrammatic representations there is necessary some compromise as a sacrifice to confusing detail. In the first stage considered (f. 14a) the tension zone is little more than a vague layer of cells (*b*) somewhat crowded between the digestion zone *a* and the thin walled but growing endosperm, *b'*. With a slight advance in age, it becomes evident that the inner part of zone *b* is giving cells to be digested, and these then become part of zone *a'*. At the same time, zone *b'* becomes narrowed down by the advancing zone *c* (f. 16), endosperm in which reserve cellulose is being laid down. This results in crowding the cells in which divisions are taking place into a narrower compass, and they occupy a region whose position is approximately represented by the narrow cross-hatched line between *b* and *b'*. It will be observed that the actual histological results of tension are seen at first in zone *b* while in later stages they pass over into zone *b'*, as represented in the diagrams. That is to say, thin-walled endosperm cells entirely disappear, but are represented by cells which are secondarily transversely divided and at the same time somewhat secondarily thickened. Zone *c'* disappears because tannin does so. This fact is represented in the diagrams by the line of demarkation overtaking, and becoming coincident with, the inner limit of zone *c'*.

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[31] A large increase in the starch content of the parenchyma beneath the epithelium was also noted.

In endeavoring to understand clearly the course of events it is necessary to keep in mind the directions of growth (1) of the endospermic plug tissue overlying the base of the embryo, and which thickens independently of the main mass of endosperm; (2) of the embryo, which is growing in thickness and length, on the one hand; and (3) of the main body of endosperm, expanding radially in all directions toward the inner integument as a limit. The enlarging endosperm cells, namely those in the interior, push against the dividing endosperm cells, which are peripherally placed. These occupy a position, therefore, between the inner integument and the inner part of the endosperm, the cells of which are enlongating radially. These thin walled, dividing cells constitute a tension zone. But in the region of the embryo, the digestive zone takes the place of the inner integument, so that the tension zone, where the secondary division therefore are taking place, dips inwardly. The plug tissue however is also in division, and this independent disc of tension tissue meets and merges with the other at the place where it dips inwardly. The place of juncture becomes more and more obvious with age till the completion of secondary cell-wall thickening, when it remains visible on account of the irregularly matched cells. This is a plane of weakness where, during the initial stage of germination, the endosperm plug is released.

The constant advance of the definitive endosperm opposed to the growing embryo results in the reduction of the zone in question. I have already pointed out that the secondary divisions in the endosperm cells which characterize it are always parallel to the integuments. They are therefore at right angles to the axis of the embryo. If these are taken as the criterion of the zone, it is seen that it is at last reduced to a thin layer of cells (*b′*, f. 19, 20), which are able neither to take on the definitive character of endosperm cells, nor entirely to resist the influence of the embryo. This is shown by the partial thickening of the walls, and in the partially digested condition of the contents. The shearing caused by the opposed directions of growth is also to be seen in the oblique and more or less distorted forms of the cells (f. 20, 21).

*Tannin in the zones of digestion.*—It has been shown that during the third period of development tannin figures prominently in the endosperm in connection with the laying down of reserve cellulose. It has been pointed out, however, that the reaction is not confined to the cells alone which are active in this regard, that, in a word, it occurs everywhere in the endosperm, the statement being intended to include the zones of digestion. For example, ethyl nitrite material thirteen weeks after pollination showed that the tannin in the tension tissue and in the digestion zones proper is in marked amount, as it is also in the embryo. This is true of younger stages, and of more advanced ones until the condition is reached represented by figure 18, when, along with the general reduction of tannin throughout the endosperm, the amount in the digestive zones is also reduced. This disappearance is not synchronous with that in the embryo, in which tannin is discoverable for a longer period. It must also be added that the tannin in the cells in these zones, is not confined to the cell walls as it is elsewhere in the endosperm. This, with the earlier disappearance of tannin in them than in the embryo, may be taken to indicate that the tannin here is concerned chiefly with the embryo.

*Digestion of Reserve Cellulose.*—As reserve cellulose plays, if any, a very inconsiderable part in the nutrition of the embryo and because it has been studied, first by Sachs, and very carefully by Reiss (1889), both histologically and chemically, it has been no part of my purpose to concern myself with it. I can find during embryogeny no evidence that there is any digestion of reserve cellulose, even during the short period when this may be suspected to occur. On the contrary, as I have already pointed out, the process of cell wall thickening proceeds inwardly toward the embryo until the resting condition is entered. One point of disagreement, however, as between Sachs and Reiss has been looked into by me, namely, the presence or absence of the optical evidence of the primary membrane in the definitive endosperm cells. Sachs says: ". . . die primären Zellhäute sind leicht als doppelte conturirte Lamellen zwischen den Verdickungschichten zu erkennen"[32] (1862, p. 242). This Reiss denies categorically, thus: "Die Wände lassen keinerlei Schichtung, auch keine Mittellamelle sichtbar hervor." The fact appears to be that the observation is not as easy as Sachs' wording would lead one to expect. While not everywhere visible with equal ease, however, there is no very great difficulty in determining the substantial correctness of his statement. As for the manner of digestion of the reverse cellulose, I am able to verify Reiss' account.

## Origin of the Ferments.

Sachs believed that the digestion of the endosperm in the date is due to a ferment secreted by the endosperm. Were this not so it would be difficult to explain the remarkable coincidence that the softening of the endosperm exactly corresponds to the growth of the haustorium. "Dieser Umstand macht es eher wahrscheinlich, dass die Epithel einen Stoff an die nächsten Endospermzellen abgiebt, der die Lösung des Zellstoffs bewirkt."

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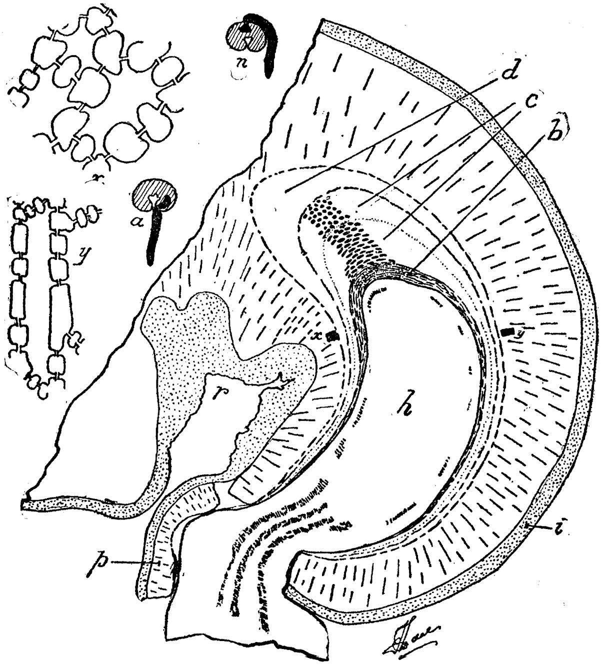
[32] Quoted by Reiss.



B. PHOENIX DACTYLIFERA.

**Transverse section through a germinating seed in which the embryo has formed in an abnormal position.—*h*, Haustorium. *i*, Integuments. *p*, Endosperm plug which caps the embryo during the resting stage. *r*, Raphe, partly torn out. *x*, *y*, Portions of endosperm the cells of which are represented on a larger scale (the same for both) at the left. The relative position of this abnormally placed embryo and of a normally placed one is shown at *n* and *a*.**

Nevertheless, Sachs' conclusion was not accepted without demur. Grüss (1896) especially thought he had proved to the contrary, though his evidence is not convincing. Newcombe (1899) showed that a cytohydrolytic enzyme could be extracted from the endosperm, and he thought that it was present "in the softened layer of endosperm against the cotyledon" but it is clear from the context that he did not believe the enzyme to originate in the endosperm but in the cotyledon. The point however being moot, Pond (1906) showed conclusively that cytase does not originate in the endosperm.

Sachs' reasoning is thus justified. It is a well-known fact, however, that the outline of the zone of digestion is not parallel to the surface of the growing haustorium, and while this does not vitiate the reasoning it calls for explanation. Figure 25 shows that the digestion begins at the end of the cotyledon, where the endosperm cells are isodiametric. It is clear that there is a localization of enzyme secretion in this region of the cotyledon. The movement of the ferments into the endosperm from this on must be due either to the anatomical conditions in the endosperm, or to a continuation of the localization of more active ferment secretion. If one follows the movement of the haustorium, one sees that it is not related to the shape or position of the cells of the endosperm (text f. B). Its more rapid growth may be parallel to or athwart the longitudinal axes of the cells, and is not always the same in amount and direction in the regions where the cells are approximately isodiametric. There is no evidence, moreover, that there is any more usable passage-way through the endosperm by virtue of thinner walls or more pores. We must conclude, therefore, that the shape of the digestion cavity is in large part determined by the localization of greater activity of ferment secretion in the haustorium. We are urged to the conclusion, further, that the greater secretion of ferments is at first at the end of the cotyledon, but that it becomes relatively less active, the scene of greater activity passing over to the sides of the haustorium. In this connection, the finding of a seed in which the embryo had suffered abnormal displacement, so that it lay in a position almost antipodal to the normal one, is of interest. It was discovered only after it had been germinating for some time (f. B).

The endosperm was normal in respect to its histological characters, its cells being arranged in the usual way. Presumably there was some adjustment quite near the embryo, and there is evidence for this in the configuration of the cells in the plug. Both the region of greatest growth in the haustorium and the area of most active secretion are opposite to the greatest thickness of endosperm to be digested. We cannot in this case say that this part of the haustorium is the end or the side, but it appears conclusive that the character of the endosperm cells has nothing to do with the direction of growth of the haustorium. We may, indeed, say that the behavior of the haustorium is adaptive, and that this organ adjusts its behavior adaptively when the normal conditions are disturbed, until a more satisfactory explanation is to be had.

## SUMMARY AND CONCLUSIONS.

The more important features of the foregoing account may be summarized as follows:

1. The development of the embryo, seed and pericarp with reference to nutrition have been followed from the anatomical-physiological point of view.
The embryological history has been articulated with the period of germination, and the continuity or discontinuity of the various processes involved in the digestion of the endosperm has been determined.

2. Before the time of pollination, the antipodal cells, and for some time thereafter, a tapetum are active agents in the nutrition of the embryo-sac. This first digests the small nucellus; it then becomes active at the antipodal pole, and forms a group of digestive pockets around the degenerating antipodal apparatus which ultimately form a single cul-de-sac, the function of which is to digest ovular (chalazal) tissue and to receive nutrients from the raphe. The chalaza in the mature seed is not determined by the configuration of the integuments (as is frequently the case), but by the manner of development of the endosperm cul-de-sac, which may in turn be related to the main path along which food materials pass in the raphe.

3. The earlier phase of development of the seed is marked by a longitudinal distortion which results in altering the position of the embryo, swinging it through an arc of 90°. This feature is common among the Palmae.

4. During the first three months following pollination the growth of the embryo is very slow. This period is devoted to the growth of the tissues of the ovule. The following period is marked especially by the development of the embryo and the laying down of reserve cellulose by the thickening of the walls of the endosperm cells.

5. Starch has been found to play only a brief rôle in the basal portion of the carpel and in the ovule. It is found in small and continually reduced quantities until the embryo is 6–7 weeks old. At this time traces only are found in the integument between the micropyle and the funicle. Subsequently none is found, till the embryo is about 17 weeks old, when it may appear in the radicle and in the cotyledon. Its appearance in any position may be taken as indicating a temporary reduction of growth activity in that place. This appears also to be true both of starch and of oil in the early stages following germination.

6. Certain tannins are important quantitatively and in their nutritive relations. I have distinguished for convenience between aplastic and plastic tannin. Aplastic tannin appears in particular cells and remains there permanently. Plastic tannin undergoes translocation, is consumed and disappears. This is taken as evidence of its nutritive rôle.

(a) Aplastic tannin in the carpel occurs in the hypodermis and in the idioplasts. The latter form a well marked sub-hypodermal zone which begins to be laid down at about the time of pollination. Tannin as such does not migrate into these cells, nor into the hypodermal tannin cells. There is therefore no evidence that during ripening there is a segregation of tannin, as reported for certain other fruits.

During ripening the tannin in these cells, as indeed all the aplastic tannin, becomes insoluble, and hence tasteless. There is no evidence forthcoming that this tannin is a glucoside and is oxidized, as tentatively held by Slade[33] and for which a slight amount of evidence has been mentioned by Vinson. Slade's view, however, is possibly applicable to the tannin described in the endosperm, and it would thus be a source of energy (*vide infra*).

The idioplasts form a morphological layer of cells which bends toward the ovule in the sutural region, but is here discontinuous. There is evidence here however of the presence of a small amount of tannin of translocation which becomes more and more marked in quantity toward the base of the carpel and in the tissues subjacent to the ovule, or young seed.

Aplastic tannin also occurs in a comparatively few, usually elongated, elements scattered throughout the inner mesocarp, and in a few cells also of the endocarp.

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[33] See Vinson (1907).

(b) In the ovule all the tannin is at first plastic. Its distribution is such as to indicate clearly that it is concerned in the nutrition of the embryo-sac. The period following fertilization is characterized, on the anatomical side, by the torsion of the ovule, and, on the physiological side, by the rapid growth of the chalazal end of the embryo-sac into the tanniferous tissue of the raphe. The whole of the ovular tissues, properly speaking, now contain tannin, and there seems to be little doubt that it is given over to the growing endosperm. However, there begins the individualization of cells as tannin-cells, which appear at first in the raphe and about the outer limits of the chalazal tissue. Later they appear also in the integuments, with the exception of the epidermis and an irregular hypodermal layer. This tannin is like that in the carpel, as it remains permanently in the special cells. Definitively, the integuments and raphe are completely loaded with insoluble tannin, with the exceptions noted. The precise relation between the plastic tannin and that which gradually appears in the manner described is not clear. They may be quite distinct, or the aplastic tannin may represent unused or unusable tannin side-tracked to accumulate as waste.

(c) In the endosperm there is from the time of fertilization till about the 19th week a very large amount of tannin. Toward the period between the obliteration of the endosperm utriculum and the secretion of reserve cellulose, there is a reduction of tannin, but when the reserve cellulose begins to be laid down there is at once a large increase in the amount of tannin within the same area. This tannin is to be found in the walls as well as in the lumen, and not as artefact. With the maturation of the endosperm, the tannin disappears in a manner to preclude the explanation that it is thrown out of the endosperm as waste.

Whether this disappearance is caused by oxidation, as is known to occur in the apple[34], or by its incorporation into a substance (possibly the reserve cellulose) with a more complex molecule, the evidence does not help us to decide.

(d) Tannin appears in the embryo in larger or smaller quantity throughout the whole of the time of development. It seems to be a principal nutrient during the earlier phase, for which there is the positive evidence that tannin is to be found in the sphere of embryonic influence as droplets, when it is seen nowhere else in similar quantity or appearance. None is found in the resting embryo. Aplastic tannin occurs in certain situations in the seedling, as observed by Sachs, and rightly interpreted by him, so far as we can yet see, as waste.

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[34] Lindet, cited by Kastle, 1910.

7. Oil. There is no oil in the carpel, integuments or raphe at any time.

Oil first appears in the endosperm during the process of ingrowth leading to the obliteration of the utriculum. The amount increases steadily until the utriculum is obliterated, or shortly thereafter. There is then a reduction in amount until the secretion of reserve cellulose has advanced somewhat, at a time approximately between that represented by figure 26a and that by figure 27. It then accumulates till the resting condition is reached. During germination it is digested before passing into the embryo.

In the embryo, oil has been found soon after it appears in the endosperm. There continues throughout the embryonic period a digestion of oil in the endosperm, which is carried on in the same manner as during germination. Its appearance in any part of the embryo is correlated with the relative cessation of activity in that part.

8. Digestion of the primary cell walls near the embryo occurs. This begins approximately between the stages represented by figures 14a and 15. The evidence for this conclusion is to be found in the change, effected in the cell walls, in a column of tissue opposite the cotyledonary pole of the embryo. Here the walls react to rather strong iodine (KI–I) by becoming blue. This material appears to afford but a relatively small amount of food material. The same form of digestion has been described by Green in *Livistona* during germination. In the date, however, it ceases at the entrance of the embryo upon the resting stage, or, at the latest, very soon after germination begins. During the embryonic period therefore, the primary membranes are digested, and this, as recorded by Sachs, does not occur during germination. The middle lamella appears to persist.

9. As shown by Pond, the digestive ferments are secreted by the embryo entirely. It is here shown that the secretion is localized, and is not equally active throughout the superficies of the embryo [or haustorium. But this does not wholly explain the behavior of the movement of the haustorium through the endosperm.

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## EXPLANATION OF PLATES.

Plate 15.—*Phoenix dactylifera.* 1, Portion of an ovule at about the time of pollination, showing the embryo-sac, tapetum and nucellus, just prior to the disintegration of the last named. 2, Ovule with the contiguous carpellary tissue to show the stylar canal leading to the pollen-tube guiding tissue. The occurrence of tannin in specia- quantity in this tissue in front of the micropyle is indicated by stippling, as also in the embryo-sac, tapetum and inner integument, and chalazal tissue. ' 2a, 2b, Transverse section through the ovule to show the pollen tube canal, single above and double at the funicle. 2 c–e, Transverse sections to show the stylar canal at different levels. The dots in the hypoderm represent tannin; stone cells cross-hatched. Raphide cells are seen in 2 d. 3, Embryo-sac and tapetum at this time. The remains of the nucellus are seen as a cap over the end of the embryo-sac. Digestion of the chalaza is proceeding rapidly. 4, Pit formed of contiguous thick walls of adjacent cells; the antipodal cells within this pit before degeneration of the basal cell is apparent. 4a, The basal and middle cells degenerated. 5, Ovule with one-celled embryo. Multinucleate parietal endosperm. Its activity beyond the antipodal apparatus is evident. 6, Ovule 7–8 weeks after pollination. Distribution of tannin (the more densely reacting tissues) shown by the dots; starch by cross-hatching. Deglet Noor. 7, Ovule, Deglet Noor, 8-9 weeks after pollination. The post-chalazal growth of the endosperm is seen, together with the general torsion of the ovule in the direction indicated by the arrow points. Tannin indicated by the dots and conventionalized cells. 8. Endosperm cells at ten weeks, when the oil is first seen. 9, The nuclei of the flattened endosperm cells of the superficial layer bordering the utriculum. The manner in which the oil appears with reference to the nucleus is evident.

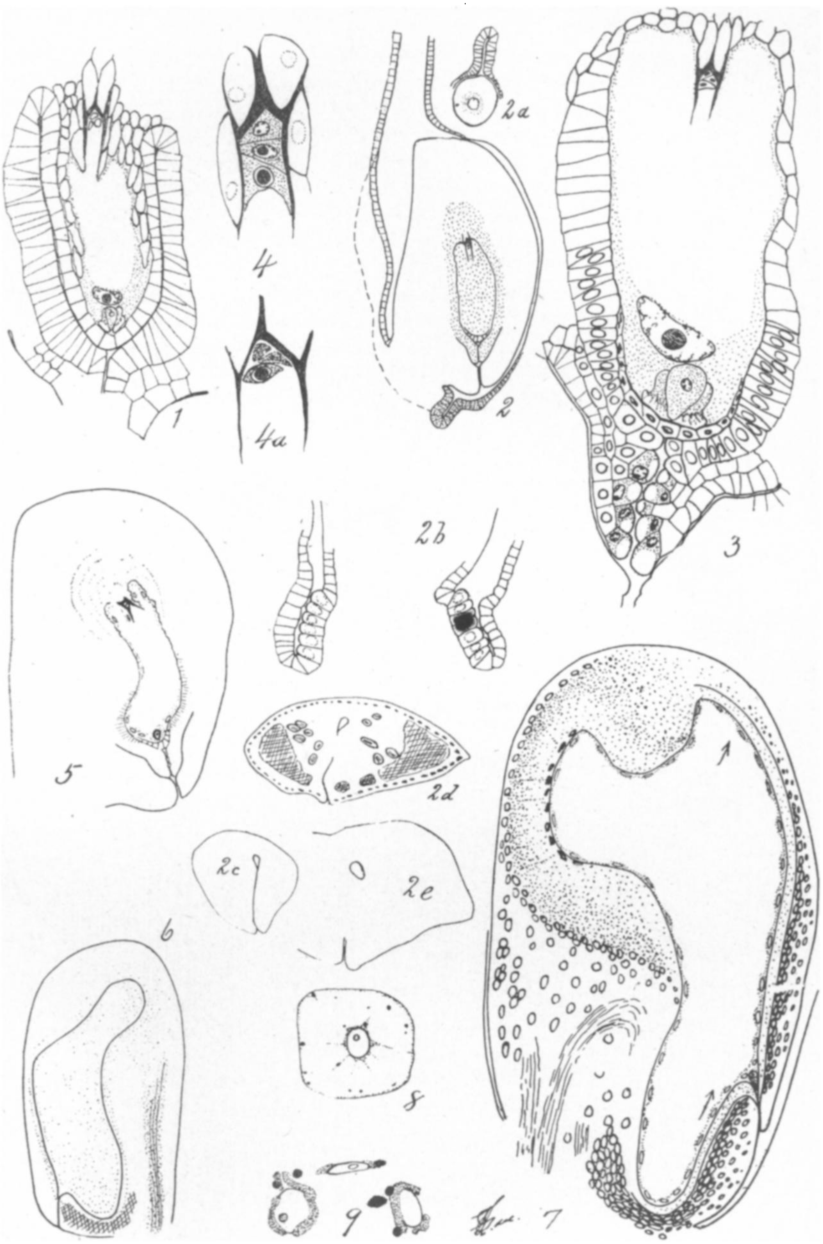
Plate 16.—*Phoenix dactylifera.*—10, (*Cf.* f. 8-9) Ovule, Deglet Noor, 11.5 weeks. Endosperm growing inwardly to obliterate the utriculum. Beginning of oil secretion. 11, Portion of transverse section of carpel and ovule to show the sutural sector in which a diffuse tannin reaction appears. 12, Longitudinal section of mature seed indicating the topography. *Ch.* chalaza. *End.* endosperm. 13, Transverse section through young seed through the chalaza. The chalazal tannin tissue proper is indicated by dots. Tannin is present however, elsewhere in the raphe and integuments. Same ovule as in f. 14. 14, Young seed at the time when the closure of the endosperm is complete. The distribution of oil about the embryo is indicated by the size of the dots. 11 weeks after pollination. 14a, The region about the embryo at about this time, in which no digestive action on the cell walls is observable; *a*, Digestive zone; *b*, Tension zone, where cell divisions occur; *b'*, Thin-walled growing endosperm. 15, Ten

weeks after pollination. Area about the embryo as seen in transverse section soon after the digestion of the cell walls is begun in $a$ and $a'$; $a$, Crushed cells empty save of oil drops; $a'$, Uncrushed cells like those in $a$; $b$, Cells in which the protoplasm is being attacked and the oil segregates; $b'$, Thin-walled endosperm between the growing endosperm cells of $b$ and those of $c$. 15a, The integuments and edge of endosperm at about this time. As yet no special tannin cells have appeared (*cf.* f. 31); *o. i.* Outer integument. *i. i.* Inner integument. 16, Thirteen weeks after pollination. For full explanation see p.—. 16a, Single endosperm cell in the course of secondary thickening taken from the position in f. 16 indicated by the black square at $x$.

Plate 17.—*Phoenix dactylifera.* 17, About (17 weeks after pollination) and 18, For full explanations of these figures see p. 142. The tannin in the embryo at the age of figure 18 is shown by the dotting, which is intended to indicate the areas of denser reaction only. About 18–19 weeks after pollination. 18a, Camera lucida drawing to show the distribution of oil globules in zones $a$ and $a'$ of figure 18, in which the same is shown diagrammatically. The irregular particles are protein. 19, Nearly mature embryo; $a$, Crushed cells ($a$, $a'$, f. 20); $b'$, Partially thickened cells ($b'$, in f. 20); $c$, Definitive endosperm. 20, Detail of f. 19 through the endosperm next the embryo; $a$, Crushed cells with minute oil droplets; $a'$, Partially crushed cells with larger oil droplets; $b'$, Partially thickened cells with transverse secondary walls, with large drops and the remains of the protoplasm. Definitive endosperm to the right of $b'$. Deglet Noor. 21, Cells of definitive endosperm which have arisen by secondary division of elongated cells which have resisted digestion. 22, The same as 20 but in front of the cotyledon, showing the final divisions of the endosperm cells and the digestion of their contents. 23, Detail to show the position of the secondary division walls in the endosperm which resists digestion. $a$. Crushed cells. $b''$ partly thickened cells with their walls at right angles to the axes of the endosperm cells from which they arose. 24, Haustorium and adjacent endosperm of seedling with extraseminal portion 2 cm. long; $a$, Crushed walls, blue with iodine; $b$, Crushed walls, not blue with iodine; $c$, Cytatic digestion advanced, oil agglomerated; $d$, Cytatic digestion beginning, oil agglomerating, protein digesting; $e$, unchanged endosperm. 25, A younger stage than 24, showing the embryonic layer blue-reacting with iodine still intact. Cytatic digestion has begun in front of the cotyledon. Dots in the embryo indicate tannin; cross-hatching, starch.

Plate 18.—*Phoenix dactylifera.* 26, Embryo and adjacent endosperm showing the distribution of tannin. 11 weeks after pollination. 26a, Transverse section of the same seed as 26, showing the areas in which the secondary thickening of the endosperm cells has begun. Here tannin is abundant, as shown by the dots. 27–29, Successively older stages showing the spread of the endosperm tissue

with thickened walls and large tannin content: 27 shows the tannin in and near the embryo; 28 shows the tannin in the embryo; 27a. Large tannin content of the endosperm cells of the chalazal cul-de-sac where the secondary thickening of the cells has gone on as in the main mass of endosperm. 30. Transverse section of seed near maturity in the endosperm of which tannin still is present in certain sectors. 31, The integuments in their definitive condition. All the cross-hatched cells contain tannin. 32, Exocarp. Diagrammatic representation of the various tissues; *e*, Epidermis; *s*, Stone cells; *p*, Parenchyma, of which a few cells are drawn here and there to show their relative sizes; *id.* tannin idioplasts. 33, Outer exocarp to show the tannin cells in the hypodermal layer, and the tannin globules in the parenchyma adjacent to the stone cells, st. (Rhars). 34, Endocarp in transverse section, *t*, elongated tannin element.



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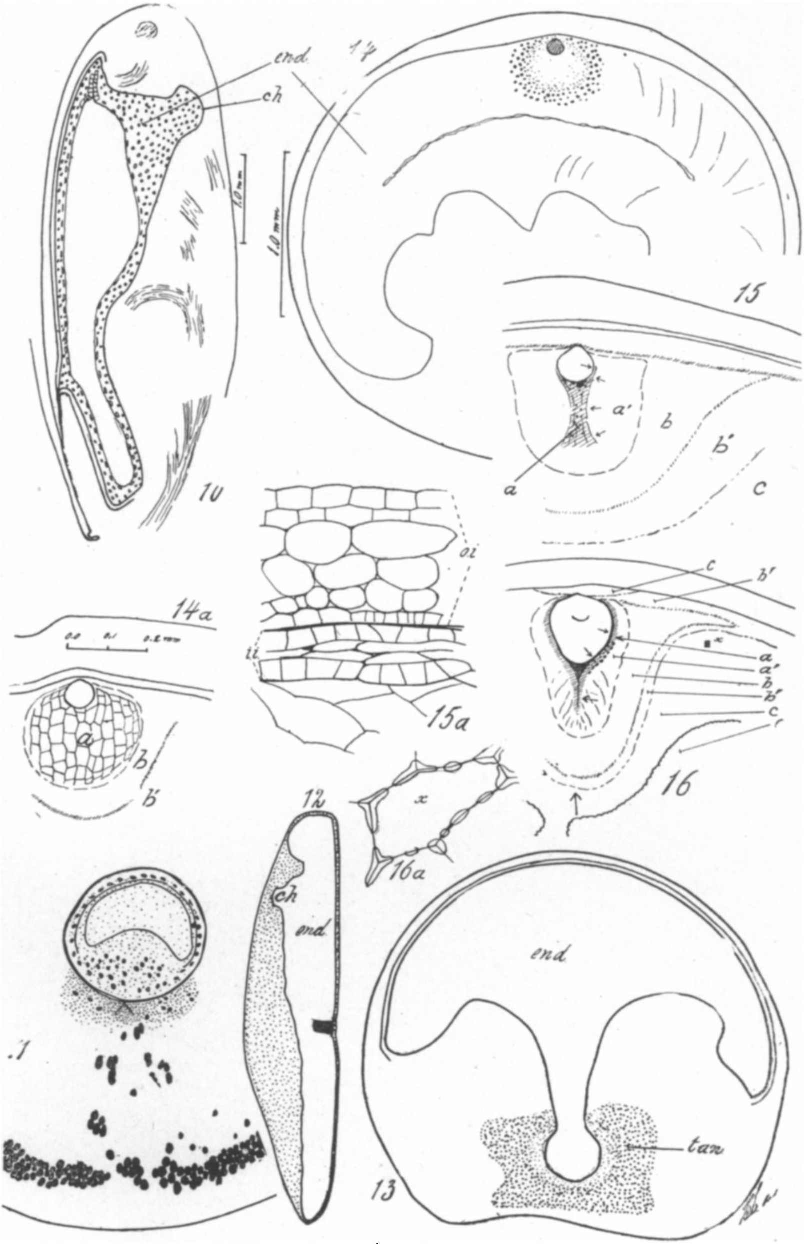

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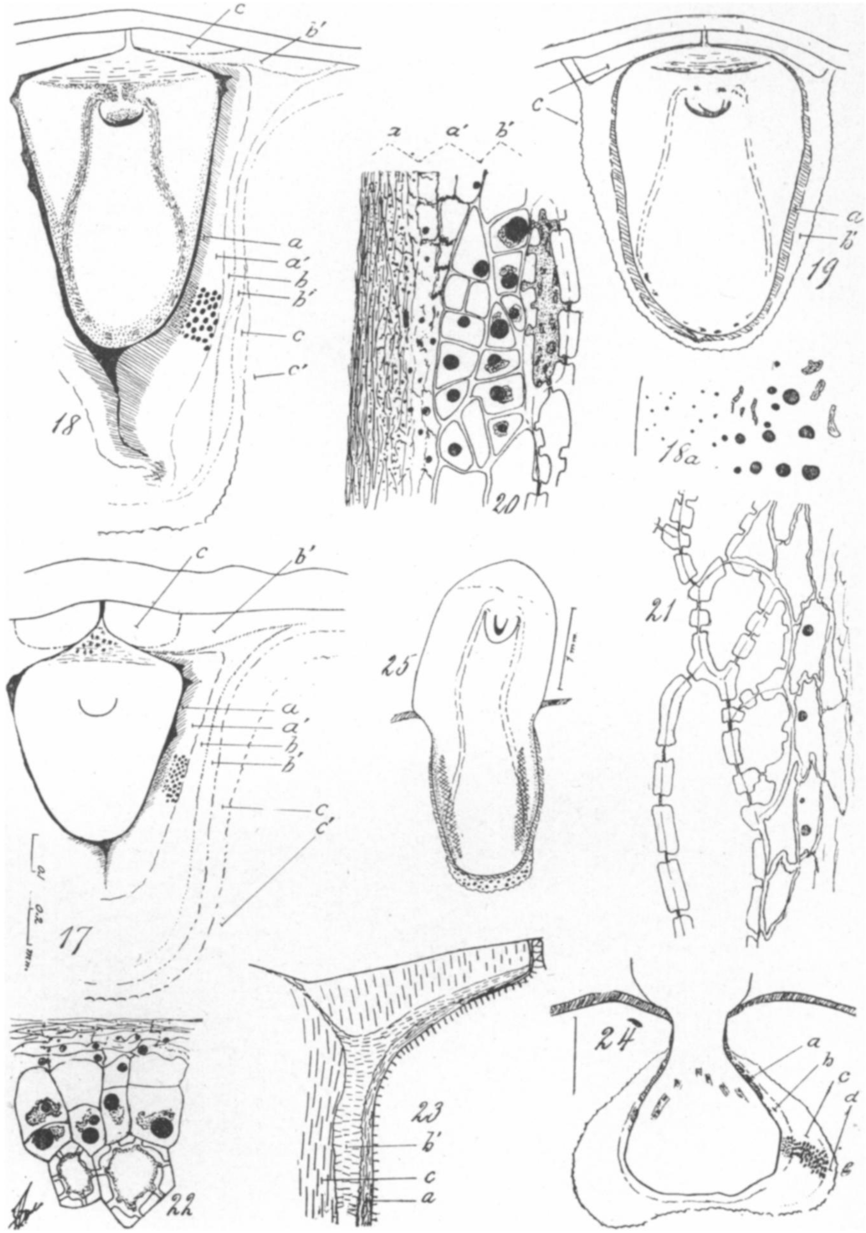

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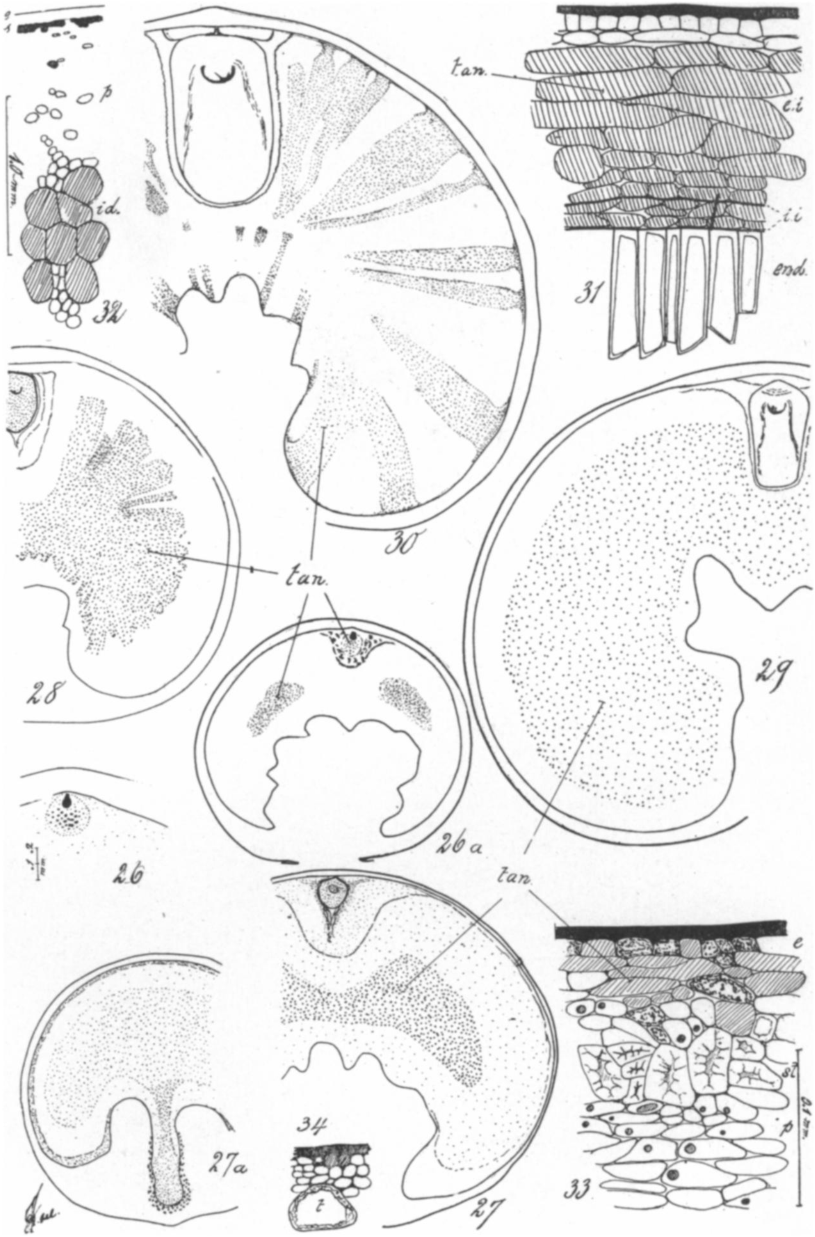

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